CHEMISTRY OF *Magnolia grandiflora* L.

By

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This paper is dedicated to my wife, Sandi, and my parents with thanks for their love and support.

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Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

CHEMISTRY OF *Magnolia grandiflora* L.

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Major Department: Medicinal Chemistry

Magnolia spp. have been investigated extensively in the past, especially for the purpose of characterizing the alkaloidal constituents which have various pharmacological activities. The chemistry and pertinent activities of *Magnolia* spp. were reviewed to set a background for the work to be described here. *Magnolia* spp. are a rich source of a variety of natural products besides alkaloids.

In the present work, a study was made of the bark, wood and leaves of *M. grandiflora* L. with special reference to the presence of sesquiterpene and lignan constituents. From the bark was isolated the sesquiterpene ketone, cyclocolorenone, for the first time from a *Magnolia* spp. Another sesquiterpene, a lactone, identified as parthenolide was isolated from the leaves and shown to be the active principle responsible for the antibacterial activity found in the leaves. The bark also yielded a novel neolignan, which was named mehonokiol, the structure of which was established by unequivocal degradative methods. Another lignoid

component, syringaresinol, was isolated for the first time from the wood of a Magnolia spp. In addition, the extract of the wood was found to be toxic to mice when administered intraperitoneally, and the active principle responsible was isolated. It was characterized as menisperine, isolated for the first time from a Magnolia spp. Two other alkaloids, anonaine and liriodenine, were also found to be present in the extract of the wood.

Cyclocolorenone, a sesquiterpene ketone with an unusual chromophoric system, a cyclopropyl-conjugated enone, is a member of the aromadendrane group. An analysis of the nmr spectral data was provided to substantiate this characterization. In support of the structure, a number of derivatives have been described.

The isolation of the sesquiterpene lactone, parthenolide, from the leaves was described. It was characterized on the basis of its spectral data as a member of the germacranolide type. This is the first report of its antibiotic activity.

A new, highly lipophilic, phenolic neolignan was isolated from the bark. It was shown to be a monomethyl ether of a bis-allylphenol known as honokiol and hence named mehonokiol. Of the two possible isomeric structures, the correct structure was deduced by degration of the tetrahydro derivatives of both to the respective n-propyl anisic acids and identification of each of these acids through unambiguous synthesis.

Syringaresinol, a lignan of the pinoresinol type was isolated from the wood of Magnolia and its structure and stereochemistry determined from spectral data.

The toxicity of the extract of Magnolia wood was described for the first time in the present work. Fractionation showed that the toxicity was due to an alkaloid. This fraction was separated into a phenolic quaternary alkaloid, which reprsented the toxic principle, and two other non-toxic alkaloids anonaine and liriodenine. Analytical and spectral data of the quaternary alkaloid showed that it was identical with the N-methyl isocorydinium cation, isolated for the first time from a Magnolia spp.

CHAPTER 1
SECONDARY METABOLISM AND THE CHEMISTRY OF *Magnolia*; PHARMACOLOGICAL ACTIVITY OF *Magnolia* EXTRACTS AND COMPONENTS

The Southern Magnolia, *Magnolia grandiflora* L., in itself practically a symbol of the Old South, belongs to the *Magnoliaceae*, a family which has been variously classified under the order *Ranales* or *Magnoliales*.[1] The chemistry of this very primitive plant has been studied extensively, particularly the alkaloids. *Magnolia* species which have undergone chemical investigation in this and other studies are summarized in Table 1.1 according to the classification of J. E. Dandy.[2]

Biogenetic Classification of Secondary Metabolites

Within the last half-century, fundamental studies of secondary metabolism have led to a biogenetic classification of secondary metabolites. This has led to such concepts as the biogenetic isoprene rule, the acetate-malonate hypothesis, and the shikimate pathway[3] which, in their present form, are of great predictive value in ascertaining the structure of a metabolite produced by an organism. Some discussion of this classification is of value in structuring the chemistry of *Magnolia* to be described in this dissertation.

Terpenoids of Genus *Magnolia*

The terpenoids are compounds arising from mevalonic acid-derived isoprenes, such as isopentenyl pyrophosphate, by catenation

Table 1.1 Species of *Magnolia* reported in the chemical literature

Subgenus *Magnolia*

Sect. *Gwillimia* (Asia)
- *M. coco* DC.

Sect. *Lirianthe* (Asia)
- ---

Sect. *Rytidospermum* (Asia and America)
- *M. Ashei* Weatherby
- *M. macrophylla* Michx.
- *M. obovata* Thunb. (syn. *M. Hypoleuca* Sieb. et Zucc.)
- *M. officinalis* Rehd. et Wils.
- *M. rostrata* W.W. Smith
- *M. tripetala* L. (syn. *M. umbrella* Desr.)

Sect. *Oyama* (Asia)
- *M. parviflora* (syn. *M. Sieboldii* K. Koch)
- *M. Sieboldii* K. Koch (syn. *M. parviflora* Sieb. et Zucc.)
- *M. sinensis* Stapf. (syn. *M. globosa* var. *sinensis* Rehd. et Wils.)

Sect. *Magnoliastrum* (America)
- *M. virginiana* L. (*M. glauca* Thunb.)

Sect. *Theorhodon* (America)
- *M. grandiflora* L.
- *M. Schiediana* Schlect.

Sect. *Gymnopodium* (Asia)
- *M. kachirachirai* Dandy (syn. *Michelia kachirachirai* Dandy)

Sect. *Maingola* (Asia)
- ---

Subgenus *Pleurochasma*

Sect. *Yulania* (Asia)
- *M. Campbelli* Hook f. et Thoms.
- *M. denudata* Desr. (syn. *M. Yulan* Desf.)

Sect. *Buergeria* (Asia)
- *M. Kobus* DC.
- *M. salicifolia* Maxim.
- *M. stellata* Maxim.

Table 1.1--continued

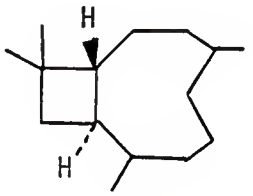
Subgenus Pleurochasma (continued)
Sect. Tulipastrum (Asia and America)
M. acuminata L.
M. cordata Michx.
M. liliflora Desr.

Note: Species cited in this work but not classified by Dandy: M. Fargesii (syn. Michelia Fargesii Andr.), Magnolia fuscata Andr. (syn. Michelia fuscata Blume, M. Figo Spreng.), M. Lennei Topf. (syn. M. Soulangeana var. Lennei Rehd.), and M. mutabilis Regel. M. X Soulangeana Soul. is M. denudata x M. liliflora.

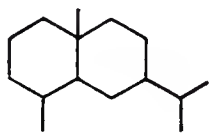
and pursuant cyclizations. The carbon skeletons thus obtained may be further modified by rearrangements: the structures of the resultant molecules are in many cases determined by the conformations of the immediate precursors and by the stereochemical requirements of the cyclizations and subsequent rearrangements.

Depending on the number of isoprenoid units involved, they are classified as mono-, sesqui-, di-, tri-, and sesterterpenes with 10, 15, 20, 30, and 40 carbon atoms, respectively.

Chemical investigation of the terpenoids of Magnolia has not been extensive, as most of the emphasis has been placed upon isolation and identification of the alkaloids. A number of more or less ubiquitous compounds and several sesquiterpenes of more restricted occurrence have been isolated. These sesquiterpenes are of the caryophyllane 1.1, eudesmane 1.2, aromadendrane 1.3, and germacrane 1.4 skeletal types.



1.1



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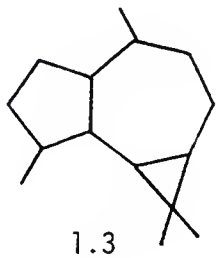

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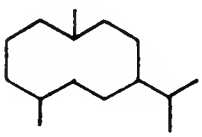

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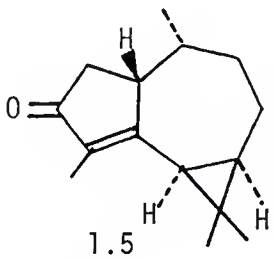

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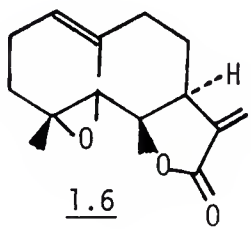


1.6

In the present work the first isolation from any Magnolia of the sesquiterpene ketone, cyclocolorenone 1.5 is reported. This cyclopropyl-conjugated enone is a member of a class of tricyclic compounds having the aromadendrane skeleton 1.3. In addition, it was found in the course of this work that the extract of the leaves possessed antibiotic activity. The compound responsible for this activity was isolated and found to be parthenolide 1.6, a cytotoxic sesquiterpene of the germacranolide class, based upon the germacrane skeleton 1.4. This compound has previously been isolated from the shrub Michelia champaca L.,[4] Michelia lanuginosa,[5] and is known to occur also in Magnolia grandiflora.[6]

In Table 1.2 are presented the terpenoid compounds of various types which have been isolated from genus Magnolia.[7-13]

Alkaloids of Genus Magnolia

Studies on the alkaloids of Magnolia have accounted for the bulk of the chemical and pharmacological literature of the genus and have been reviewed by Tomita and Nakano.[14]

A biogenetic definition of the alkaloids might state that they are nitrogeneous secondary metabolites derived from amino acids. The benzylisoquinoline and aporphine alkaloids characteristic of Magnolia spp. are derived from phenylalanine. The formation of reticuline 1.7 and magnoflorine 1.8, examples of these classes of general distribution throughout the Magnoliaceae are detailed in Figure 1.1.[15-46]

Table 1.2 Compounds of mevalonate origin reported in the genus Magnolia

Compound	Class	Species
citral	monocyclic terpene	M. obovata (lvs)[a,7] M. Kobus (lvs, stms)[8] M. salicifolia (lvs, stms)[8,9]
limonene	monocyclic terpene	M. obovata (lvs)[7] M. Kobus (bds)[10]
cineole	monocarbocyclic terpenes	M. Kobus (lvs, stms)[8] (bds)[10] M. salicifolia (lvs, stms)[8]
α- and β-pinenes	bicyclic terpenes	M. obovata (lvs)[7] M. Kobus (lvs, stms)[8]
bornyl acetate	bicyclic terpene	M. obovata (lvs)[7]
camphene	bicyclic terpene	M. obovata (lvs)[7]
camphor	bicyclic terpene	M. Kobus (bds)[10]
caryophyllene	bicyclic sesquiterpene	M. obovata (lvs)[7]
caryophyllene epoxide	bicarbocyclic sesquiterpene	M. obovata (lvs)[7]
α-, β- and γ-eudesmols	bicyclic sesquiterpene (eudesmane type)	M. obovata (lvs)[7]
cyclocolorenone	tricyclic sesquiterpene (aromadendrane type)	M. grandiflora[b]

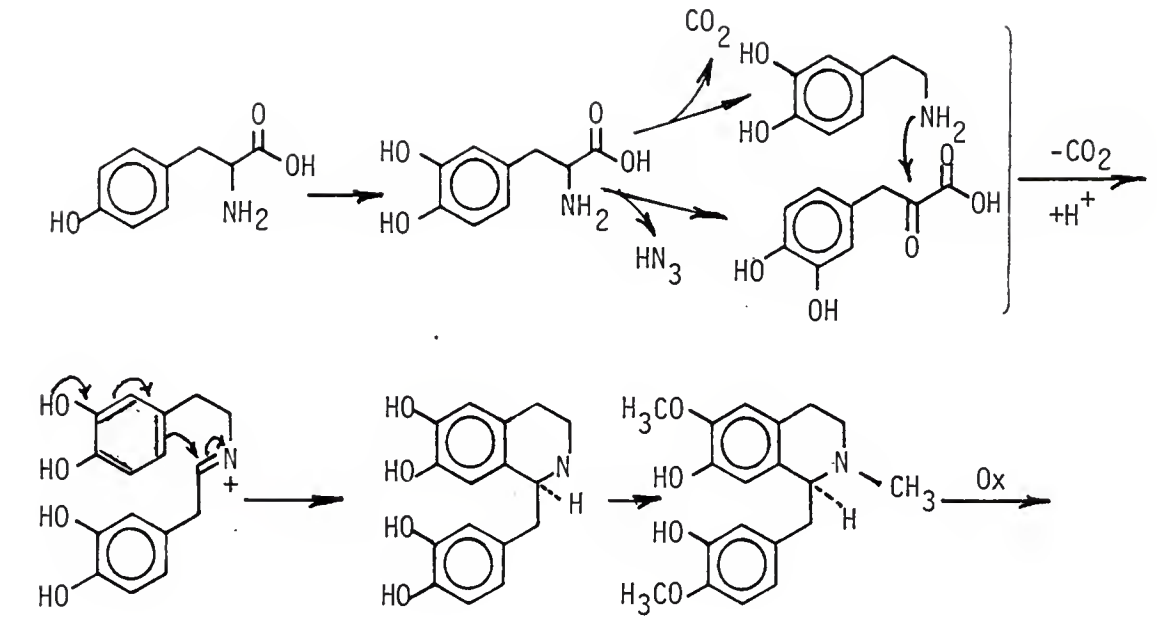
Table 1.2--continued

Compound	Class	Species
costunolide	monocarbocyclic sesquiterpene lactone (germacranolide)	M. acuminata (rt-bk)[11]
parthenolide	monocarbocyclic sesquiterpene lactone (germacranolide)	M. grandiflora (lvs)[b,6] (stms)[6]
crytomeridiol	bicyclic sesquiterpene	M. obovata (bk)[12]
campesterol	tetracyclic triterpene (sterol)	M. obovata (lvs)[7]
cholesterol	tetracyclic triterpene (sterol)	M. obovata (lvs)[7] M. grandiflora (bk)[c]
β-sitosterol	tetracyclic triterpene (sterol)	M. acuminata (rt-bk)[11] M. Campbelli (bk)[13] M. grandiflora (bk)[c]
stigmasterol	tetracyclic triterpene (sterol)	M. obovata (lvs)[7] M. grandiflora (bk)[c]
α-amyrin	pentacyclic triterpene	M. obovata (lvs)[7]
lupeol	pentacyclic triterpene	M. ovovata (lvs)[7]

[a]bk = bark, lvs = leaves, htwd = heartwood, rts = roots, stms = stems, bds = buds, wd = wood, flwr = flowers, sds = seeds.

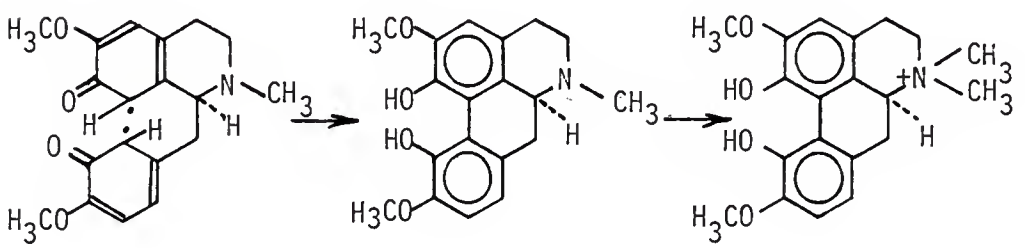
[b]This work.

[c]In the course of this work, materials with the same tlc behavior and glc retention times (on one column) as commercial samples of the above were observed.



(+)-reticuline

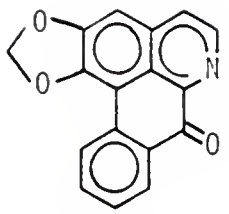
1.7



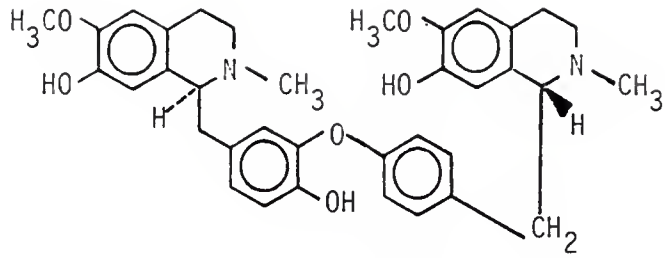
magnoflorine

1.8

Figure 1.1 Probable derivation of Magnolia alkaloids magnoflorine and reticulene from tyrosine



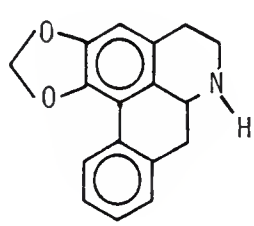
1.9



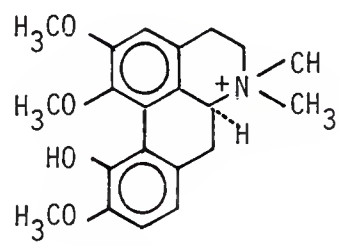
1.10

Further oxidative processes can convert these alkaloids to oxoaporphines such as liriodenine 1.9 and to dimeric alkaloids such as magnoline 1.10. The known alkaloids of Magnolia are presented in Table 1.3.

In this work are reported the isolations of anonaine 1.11; menisperine (N-methylisocorydine, 1.12), and lirodenine 1.9 from the wood of Magnolia grandiflora. Although anonaine has previously been isolated from this plant,[40] this is the first reported isolation of menisperine from any of the Magnoliaceae. This quaternary aporphine was also found to account for the toxicity of the wood extract. Liriodenine has previously been reported to be present in the leaves and wood of M. grandiflora,[40] and, in the course of this work, it was also observed in the bark.



1.11



1.12

Table 1.3 Alkaloids isolated from *Magnolia* spp.

Compound	Occurrence
phenylethylamine	
candicine	*M. grandiflora* (bk)[15] (rts)[16]
salicifoline	*M. acuminata* (stms)[17] *M. coco* (stms)[18] *M. denudata* (bk)[19] *M. grandiflora* (rts)[16] (bk)[a,21] *M. Kobus* (bk)[22,23] *M. liliflora* (bk, rts, wd)[24] *M. salicifolia* (bk)[25] *M. stellata* (bk)[26]
benzylisoquinoline	
magnococline	*M. coco* (stms)[27]
N-norarmepavine	*M. kachirachirai* (htwd)[28,29]
reticuline	*M. obovata* (rts)[30]
benzylisoquinoline (quaternary)	
magnocurarine	*M. acuminata* (stms)[17] *M. denudata* (bk)[19,20] *M. fuscata*[b] (lvs, bk)[33] *M. liliflora* (bk)[24] *M. obovata* (rts)[30] (bk)[34,35] (htwd)[35] *M. officinalis* (htwd)[36] *M. parviflora* (bk)[37] *M. salicifolia* (bk)[25]
bisbenzylisoquinoline	
magnolamine	*M. fuscata*[b] (lvs)[33,38,39] (bk)[33]
magnoline	*M. fuscata*[b] (lvs)[38]
aporphine	
anonaine	*M. grandiflora* (wd)[c,40] (lvs)[40] *M. obovata* (lvs)[30] (rts)[30] (htwd)[35,41]

Table 1.3--continued

Compound	Occurrence
aporphine (continued)	
anonaine acetamide	M. obovata (htwd)[41]
anolobine	M. acuminata (rt-bk)[9] M. coco (stms)[27,42] (rt-bk)[43] M. grandiflora (wd)[40]
asimilobine	M. obovata (lvs, rts)[30]
glaucine	M. kachirachirai (htwd)[18,29] M. obovata (lvs)[30]
N-norglaucine	M. kachirachirai (htwd)[29]
N-nornuciferine	M. grandiflora (wd)[40]
obovanine	M. obovata (lvs)[30]
aporphine (quaternary)	
N,N-dimethyllindcarpine iodide	M. acuminata (rt-bk)[9]
magnoflorine	M. acuminata (lvs, stms)[e] M. coco (stms)[18] M. denudata (rts)[44] M. fuscata[b] (lvs, bk)[33] M. grandiflora (bk)[15,20,21] M. kachirachirai (htwd)[18,29] M. Kobus (bk)[22] M. parviflora (bk)[37]
menisperine	M. grandiflora (wd)[c]
proaporphine	
stepharine	M. coco (stms)[27,42] (rt-bk)[43]
7-hydroxyaporphine	
michelalbine (norushinsunine)	M. obovata (htwd)[35]

Table 1.3--continued

Compound	Occurrence
7-oxotetradehydroaporphine	
lanuginosine	M. Campbelli (bk)[11]
liriodenine (oxoushinsunine)	M. Campbelli (bk)[11] M. coco (stms)[18] M. grandiflora (bk)[c] (wd)[c,40,45] (lvs)[40] M. mutabilis (lvs)[11] M. obovata (lvs, rts)[30] (htwd)[35,41] (bk)[35]
oxoglaucine	M. kachirachirai (htwd)[29]
oxolaureline	M. X Soulangeana[46]

[a]Absence of salicifoline in the bark of M. grandiflora var. lanceolata Ait. has been noted by Tomita et al.[20]

[b]Now considered by most authors to be Michelia fuscata Blume,[31] or Michelia Figo Spreng[31,32]

[c]This work

[d]Not isolated; identified by paper chromatography[17]

Liriodenine is of especially frequent occurrence in these plants, anonaine also being common, especially in those plants containing liriodenine. The quaternary alkaloid menisperine, however, is reported in only one of the Annonaceae, and in none of the Magnoliaceae prior to the present work.

Compounds Derived from Shikimic Acid

A major group of secondary metabolites of plants consists of phenylpropanoid derivatives, ultimately arising via shikimic acid, with the amino acids phenylalanine and/or tyrosine as immediate precursors. The members often bear as the stigma of their origin a 4-, 3,4- or 3,4,5-oxygenation pattern on the aromatic ring. Shikimic acid-derived compounds isolated from Magnolia are listed in Table 1.4.[8-10,47-59]

Oxidative juncture of cinnamyl alcohol and its derivatives at the β-positions of the 2-propenyl side chains results in a class of compounds Haworth has termed the lignans.[60,61] Oxidatively coupled phenolic compounds also appear in lignins, the polymeric phenylpropanoids which constitute the "glue" that binds wood cellulose.

To date, several hundred lignans have been isolated from the Gymnospermae and class Dicotyledonae of the Angiospermae. A summary of skeletal types of the classical lignans is presented in Figure 1.2.

More recently, the collective name "lignoids" has been proposed for the dimeric phenylpropanoids. Those derived from two units of cinnamic acid, two units of cinnamyl alcohol, or one of each, retain the "lignan" designation, whereas those formed from two units of allylbenzene, two units of propenylbenzene, or one unit of each are

Table 1.4 Shikimic acid-derived compounds of *Magnolia* spp.

Compound	Structural Type	Occurrence
anisaldehyde	benzaldehyde	*M. salicifolia* (lvs, stms)[8]
eugenol	allylphenol	*M. Kobus* (lvs, stms)[8]
methylchavicol	allylphenol	*M. Kobus* (lvs, stms)[8]
methyleugenol	allylphenol	*M. salicifolia* (bds)[10]
safrole	allylphenol	*M. salicifolia* (bds)[10]
trans-anethole	propenylphenol	*M. salicifolia* (lvs, stms)[8,9]
trans-asarone	propenylphenol	*M. salicifolia* (bds)[10]
syringin (syringoside)	3-hydroxypropenylphenol glycoside	(lvs, stm-bk) of:[48] *M. acuminata* *M. Campbelli* *M. denudata* *M. grandiflora* *M. Kobus* *M. liliflora* *M. macrophylla* *M. obovata* *M. parviflora* *M. salicifolia* *M. X Soulangeana* *M. stellata* *M. tripetala* *M. Wilsonii* *M. grandiflora* (wd)[49]

Table 1.4--continued

Compound	Structural Type	Occurrence
magnolidin	glycosidic ester of a cinnamic acid	M. grandiflora[15,49,50]
α-tocopherol and 4 unidentified chromanols	chromanol	M. X Soulangeana (lvs)[51]
magnolioside	coumarin glycoside[a]	M. macrophylla (bk)[52]
acuminatin	neolignan--bis(allylphenol)	M. acuminata (rt-bk)[9,53]
honokiol	neolignan--bis(allylphenol)	M. obovata (bk)[12,54,55] M. officinalis (bk)[54,55] M. tripetala (bk)[55] M. grandiflora (sds)[56] M. rostrata (bk)[53]
magnolol	neolignan--bis(allylphenol)	M. obovata (bk)[12,54,55] M. officinalis (bk)[54,55] M. tripetala (bk)[55] M. virginiana (bk)[55] M. grandiflora (sds)[56] M. rostrata (bk)[57]
mehonokiol	neolignan--bis(allylphenol)	M. grandiflora (bk)[6] (sds)[56]
calopiptin	neolignan--galgravin type	M. acuminata (rt-bk)[9,53]
galgravin	neolignan--galgravin type	M. acuminata (rt-bk)[9]

Table 1.4--continued

Compound	Structural Type	Occurrence
veraguensin	neolignan--galgravin type	M. acuminata (rt-bk)[9,53]
sesamin	lignan--pinoresinol type	M. mutabilis (lvs, stms)[13]
eudesmin	lignan--pinoresinol type	M. Fargesii (flwr-bds)[58]
magnolin	lignan--pinoresinol type	M. Fargesii (flwr-bds)[58]
syringaresinol	lignan--pinoresinol type	M. grandiflora (wd)[a]
syringaresinol dimethyl ether	lignan--pinoresinol type	M. Fargesii (flwr-bds)[58] M. Kobus (sds)[59]

[a]This work

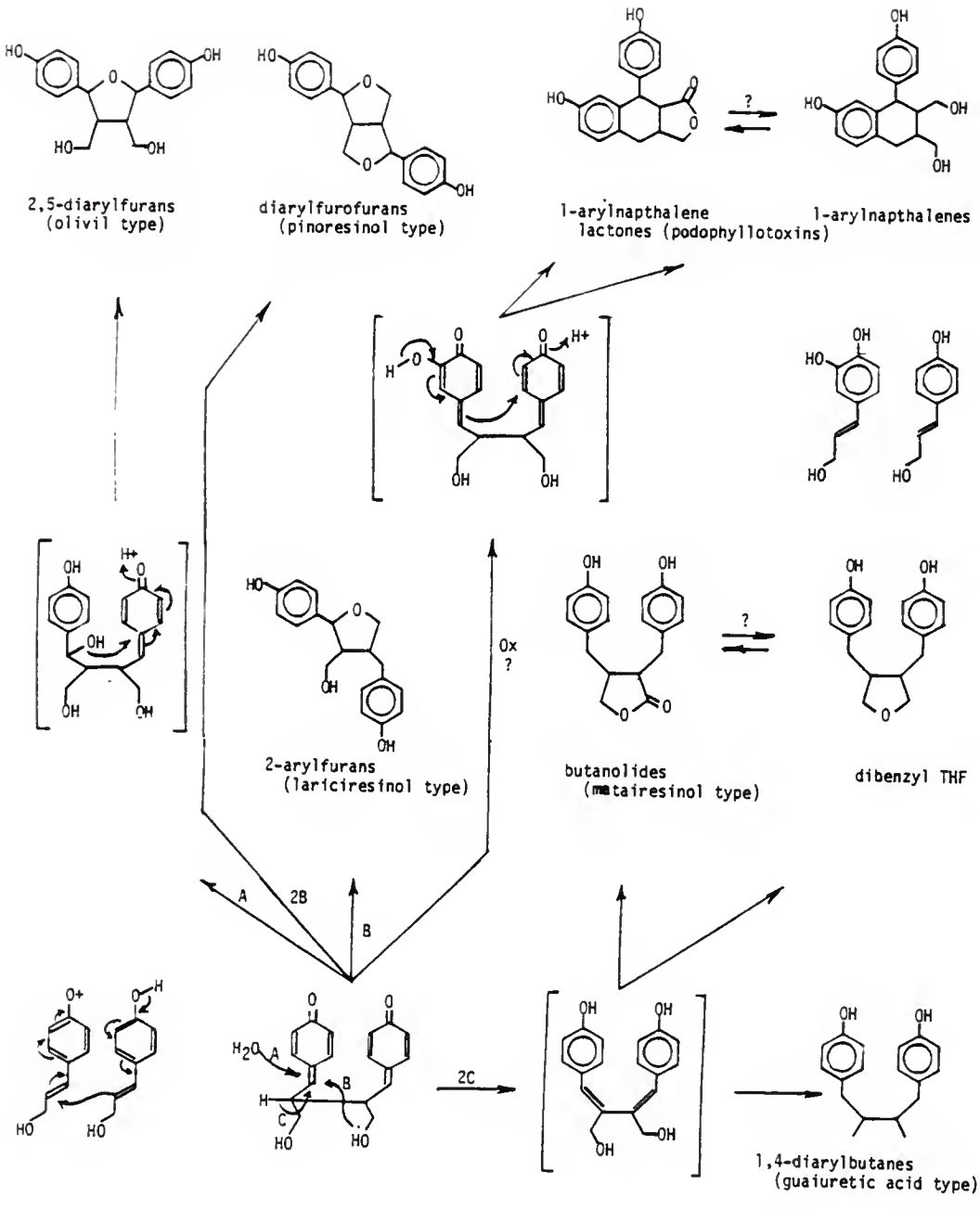
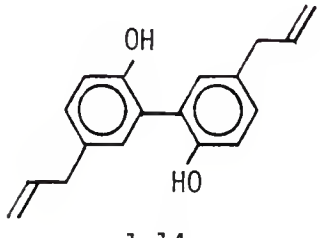


Figure 1.2 Notional representation of lignan biogenesis and structural types

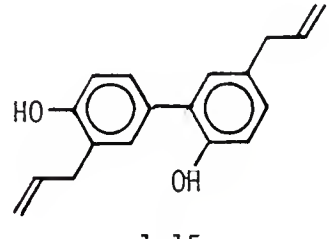
designated "neolignans."[61,62] The neolignans are more restricted in their distribution than the lignans proper, having been isolated only from two subclasses, the Magnoliidae and the Rosidae.[62] Those large groups encompass, however, a number of major families, including the Magnoliales and the Laurales.

The known lignans of Magnolia grandiflora are presented in Figure 1.3. To this list one may now add the isolation of the lignan syringaresinol 1.13, described in this work--the first reported occurrence of this compound from a member of the genus Magnolia.

One class of compounds which would now be considered as neolignans is that of the bis-allylbiphenols, which include magnolol 1.14, honokiol 1.15, acuminatin 1.16, and dehydrodieugenol 1.17. Of these, the former three have been found only in various Magnolia species (cf. Table 1.4), while the latter, isolated from Litsea turfosa Kosterm. (Lauraceae)[63] is the only bis-allylphenol-type neolignan occurring outside the genus Magnolia.



1.14



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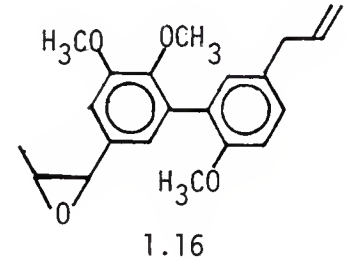
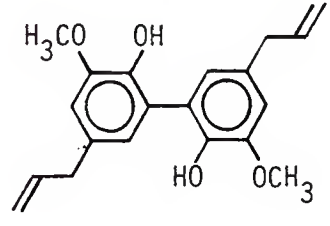
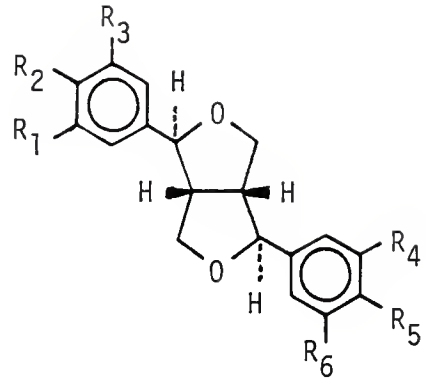


1.16



1.17



$R_1, R_2 = R_4, R_5 = OCH_2O, R_3 = R_6 = H$ sesamin

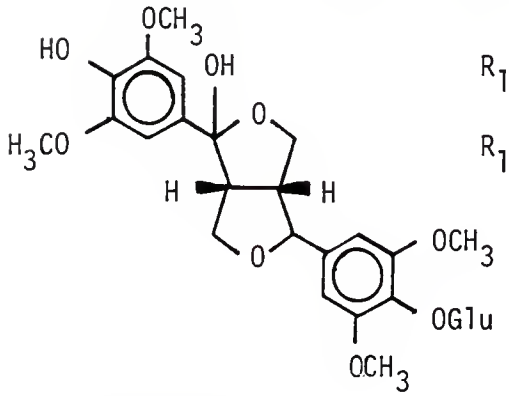
$R_1 = R_2 = R_4 = R_5 = OCH_3, R_3 = R_6 = H$ magnolin

$R_1 = R_2 = R_4 = R_5 = R_6 = OCH_3, R_3 = H$ eudesmin

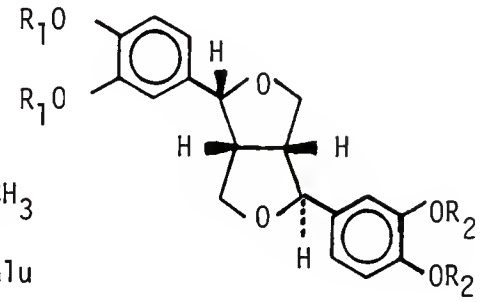
$R_1 = R_3 = R_4 = R_6 = OCH_3, R_2 = R_5 = OH$ syringaresinol 1.13

$R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = OCH_3$ syringaresinol dimethyl ether

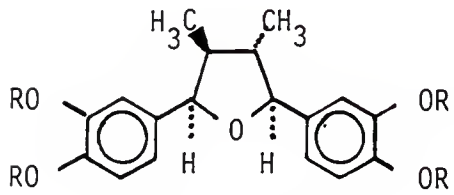
$R_1 = R_3 = R_4 = R_6 = OCH_3, R_2 = OGlu, R_5 = OH$ acanthoside B



magnolenin C

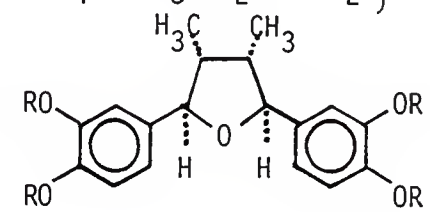


$R_1 = (CH_2), R_2 = CH_3$
$R_1 = CH_3, R_2 = (CH_2)$
} or fargesin



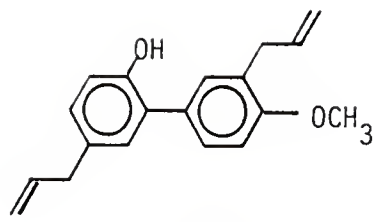
$R = CH_3$ veraguensin

$R = (CH_2)$ calopiptin

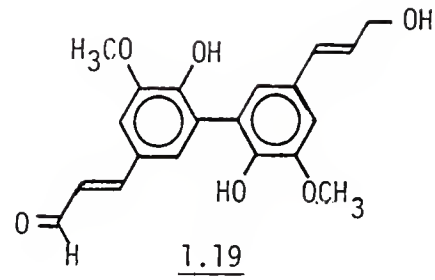


$R = CH_3$ galgravin

Figure 1.3 Known lignans of Genus Magnolia



1.18



1.19

To this class, a fifth compound, mehonokiol 1.18, may now be added, having been isolated in the course of this work from the bark of M. grandiflora.[a] The structure was unequivocally established by spectroscopic and degradative methods, followed by syntheses of the degradation products.

A sixth biphenyl, zehyerol 1.19, obtained from Zeyheria[b] montana Mart. and Z. tuberculosa (Vell.) Bur. ex Verlot (Bignoniaceae)[64] has been reported recently, although, strictly speaking, it is not a neolignan but a lignan.

A number of compounds of mixed shikimic acid-acetogenin origin such as flavonols and floral anthocyanins, have also been isolated from Magnolia species.[65-69]

---

[a]Recently El-Feraly and Li have obtained 1.18 from the seeds of this plant, in addition to magnolol and honokiol. The structure was determined by ^{1}H- and ^{13}C-nmr spectroscopy.[56]

[b]Zeyheria is spelled Zehyera by the author.

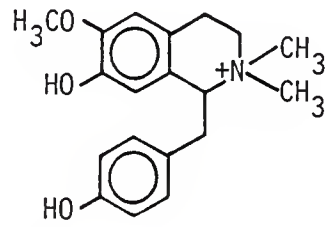
Pharmacological Activity of Magnolia Preparations and Magnolia Compounds

Much of the impetus for research upon Magnolia has been its long history of use in medicinal preparations. Historically, a number of species of Magnolia have been employed in Chinese medicine,[70] American Indian medicine,[71,72] and even listed in American pharmacopoeiae and pharmacognosy texts as bitter tonics, antimalarials, and diaphoretics.[73,74,75] The use of Magnolia extract in medicine has been proposed even as recently as 1953.[76]

A number of these crude preparations do display some pharmacological activity. A preparation from Cortex Magnoliae has been found to be bacteriostatic against Staphylococcus aureus, but not against Eschirechia coli.[77] An extract of M. Kobus has shown some antiviral activity in mice at subtoxic levels.[78,79] The extract of M. grandiflora was found to have acaricidal activity,[80] and to lower blood pressure without effect on heart action or breathing.[76]

Many of the Magnolia preparations show pronounced effect on the nervous system; some contain anticholinesterase, neuromuscular-junction (nmj) blocking or ganglionic blocking agents. The ether extract of M. obovata, at a dose of 1 g/kg intraperitoneally (IP) in mice afforded a depression of spontaneous activity and muscular weakness, characterized as a central nervous system effect. The aqueous extract, at the same dose, showed prompt respiratory paralysis.[81] The aqueous extract of the Chinese herbal Shin-I (bark of Magnolia Fargiesii) displays marked acetylcholine-like action on the frog rectus-abdominus. In addition, the authors isolated an unidentified alkaloid ($C_{17}H_{19}NO_3$) with curare-like action from the same drug.[82]

Several of the active principles of neuroactive Magnolia extracts have been isolated and identified. The bis-benzylisoquinoline, magnoline, 1.10, has a hypotensive effect and shows anticholinesterase activity.[83] Compounds associated with the observed "curare-like" effects of Magnolia extracts are magnocurarine 1.20, magnoflorine 1.8, and salicifoline 1.21.[84] Magnocurarine, the most active of these quaternary alkaloids,[84] has a ganglionic-blocking activity comparable in strength to hexamethonium bitartrate.[85] It exerts upon frogs an action similar to that of d-tubocurarine (d-TC) 1.22,[a] but of longer duration and only one-tenth as active.[87]



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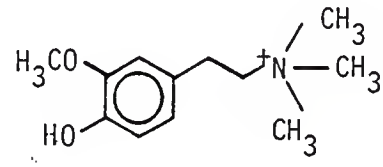

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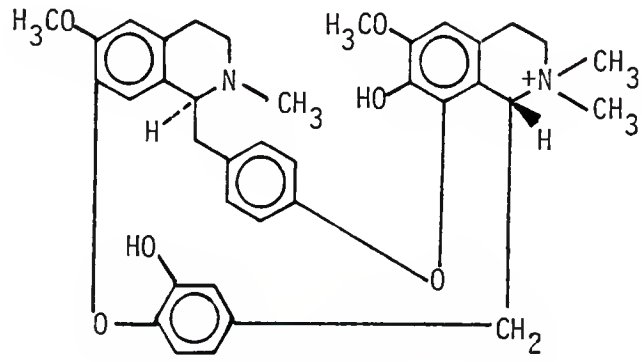
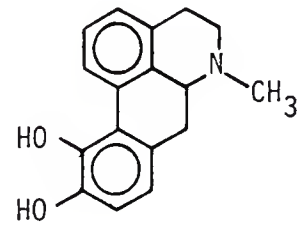


1.22[a]

[a]Previously thought to be fully quaternized, the structure of d-TC has recently been revised.[86]

In other work, on rat sciatic-skeletal muscle preparation *in situ*, these alkaloids exerted a curare-like action. The same blockade was observed with frog *rectus abdominus* (*in vitro*), except in the case of salicifoline, which caused contraction of the muscle.[85]

The noraporphine anonaine 1.11, isolated in the course of this work, has antibiotic activity at 100 mg/ml against *Staphylococcus aureus*, *Mycobacterium smegmatis*, and *Candida albicans*.[88] It also exerts a hypotensive effect in mice and rabbits[89] and has been shown to be an inhibitor of dopaminergic response, an activity associated with a number of analogs of apomorphine 1.23.[90] Its specific effect in rats is as an antagonist of dopamine-sensitive adenylate cyclase in the caudate nucleus.[90]



1.23

In these laboratories, anonaine (as the hydrochloride) showed no acute toxicity on injection in mice at a dose of 200 mg/kg (IP).

The *Magnolia* alkaloid liriodenine 1.9, also observed in the course of this work, has an antibiotic activity similar to that of anonaine.[88,91] In addition, it is an inhibitor of human carcinoma of the nasopharynx *in vitro*.[92]

The toxicity of M. grandiflora extracts has not previously been reported. In the course of this work, it was found that the aqueous fraction resulting from distribution of the extract between water and ethyl acetate is toxic at a level of 250-350 mg/kg (IP) in mice. The action is overtly curare-like, resulting in prompt respiratory arrest. The alkaloid responsible for this activity was isolated and found to be identical with menisperine 1.12 (N-methyl isocorydinium iodide), the nmj-blocking[76,93-6] and ganglionic-blocking activities[76,93,95,96] of which are well established.[a] Menisperine, then known as "chakranine," was originally described as exerting ". . . ganglionic blockade selectively exercised against the autonomous nerve pathways of the respiratory system."[93] Erhart and Soine, using the cat tongue/hypoglossal nerve system in vivo, have effectively characterized the nmj-blocking activity of this drug as very similar to that of d-TC itself.[94]

The LD_{50} of the pure iodide was determined in this work to be 10 mg/kg (21 μmol/kg, IP, mouse). For comparison, Kamat et al. obtained a value of 2.2 mg/kg (5.9 μmol/kg, IV, mouse) for the chloride.[93]

The results of a pharmacological screening procedure routinely employed in these laboratories are presented in Table 1.5.[b] These data do not indicate any definitive pattern of pharmacological effects for this compound: although the mydriasis observed is indicative of ganglionic blockade, no positive indication for nmj-blocking activity

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[a]Moisset des Espanes has reported, however, that menisperine does not have a curarizing effect, but ". . . diminishes direct and indirect contractibility and excitability in a manner resembling tetraethylammonium salts."[97] This same author observed meiosis upon intra-lymphatic injection in frogs.[98]

[b]After the method of Campbell and Richter.[99]

Table 1.5 Pharmacological evaluation of Magnolia toxic alkaloid

Sample Magnolia Bark — Date 9/19/78

Dose 12 mg/kg, 0.6 mg/ml — Time 3:08 p.m.

Animal Mice

		Animals					
		1	2	3	4	5	Final
	Paw Temp. before						NT
	Paw Temp. after						
	Body Temp. before	37.5	37.5	37.5	37.5	37.5	
	Body Temp. after	---	36	36	---	32	
	Average Wt. (g)						25
Sign: 1.	Paw Temp., +	---	---	---	---	---	
2.	Body Temp., +	↑	-	-	↑	-	
3.	Body Temp., +	|	-	-	|	-	
4.	Ptosis	|	-	-	|	-	
5.	Salivation	|	-	-	|	-	
6.	Lachrymation	|	-	-	|	-	
7.	Mydriasis	|	+	+	|	-	+
	Miosis	|	-	-	|	-	
8.	Piloerection	|	-	-	|	-	
9.	Locomotor Activity, +	|	-	-	|	-	
10.	Straub tail phenom.	|	-	-	|	-	
11.	Righting reflex (r.r.) abolished	|	-	-	|	-	
12.	Head drop (with r.r. present)	|	-	-	|	-	
13.	Pos. Haffner (with r.r. present)	|	-	-	|	-	
14.	Locomotion Activity, +	|	-	-	|	-	
15.	Abduced hindlegs (with r.r. present)	|	-	-	|	-	
16.	Unsteady gait	↓	-	-	↓	-	

Dead after 5 minutes (animal 1) / Dead after 25 minutes (animal 4)

17. Others: Onset of effects ca. 5 min., with marked depression of activity[a] and labored breathing. Moribund animals become increasingly cyanotic, and breathing more convulsively. Just prior to death, animal usually rears and/or scampers frantically. Animals not receiving a lethal dose usually recover within 30 min. At 2-3 x LD_{50}, death occurs in less than 5 minutes. At LD_{50} deaths occur at 15-20 min., with an occasional mouse succumbing more promptly.

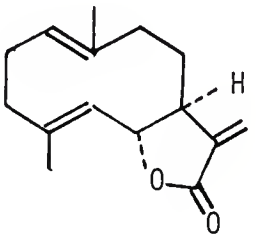
18. CMTD (mg/kg)

[a]Animals remain capable of locomotion when disturbed.

(head-drop, ptosis, strabismus)[99] other than apparent respiratory arrest is evident. However, such activity is not observed under these conditions for d-TC itself.[99]

Antibiotic activity of a very low order has been reported for menisperine (chloride): it is active against Mycobacterium pyogenes var. aureus and Streptococcus pyogenes at concentrations of 0.5 and 0.3 mg/ml, respectively.[93]

The sesquiterpene lactones parthenolide 1.6 and costunolide 1.24[100] have been shown to be inhibitors of human epidermoid carcinoma in the nasopharynx test system. In the course of this work it was established that parthenolide is responsible for the antibiotic activity observed in extracts of old, yellowed leaves of M. grandiflora.



1.24

The lignans sesamin, eudesmin, and syringaresinol 1.13, all of which occur in various species of Magnolia (cf. Table 1.4) have some antitubercular activity in vitro.[101,102]

Finally, compounds related to the diallylbiphenols found in Magnolia have been synthesized for medicinal and other applications,[103] and several have been patented for use as anticoccidial agents in poultry feed.[104]

CHAPTER 2
SESQUITERPENOIDS OF *Magnolia grandiflora* L.

Ketone from the Bark Extract

Partition of the concentrated ethanolic extract between water and ethyl acetate separated two fractions of grossly different polarities. The hydrophilic fraction contained highly polar compounds such as glycosides and quaternary alkaloids, the isolation of which has been described elsewhere.[15] Fractionation of the lipophilic components and characterization of the major principles form the subject matter for the bulk of this dissertation. A summary of the various constituents found in the bark of *Magnolia grandiflora* is given in Figure 2.1.

Thin-layer chromatographic examination of the lipophilic fraction revealed the presence of significant amounts of an ultraviolet-absorbing component which gave a positive reaction with 2,4-dinitrophenylhydrazine spray. The sample had a uv-maximum of 260 nm which was unaffected by the addition of base, thus showing that it was nonphenolic. This component was isolated by column chromatography on silica gel-cellulose (1:1) by elution with 5% acetone in benzene. The sample so obtained was, however, contaminated with phytosterols which were partially removable by precipitation from methanol at 5°C. Examination of the steroid fraction by temperature-programmed and isothermal gas-chromatography showed the presence of peaks which were identifiable with authentic

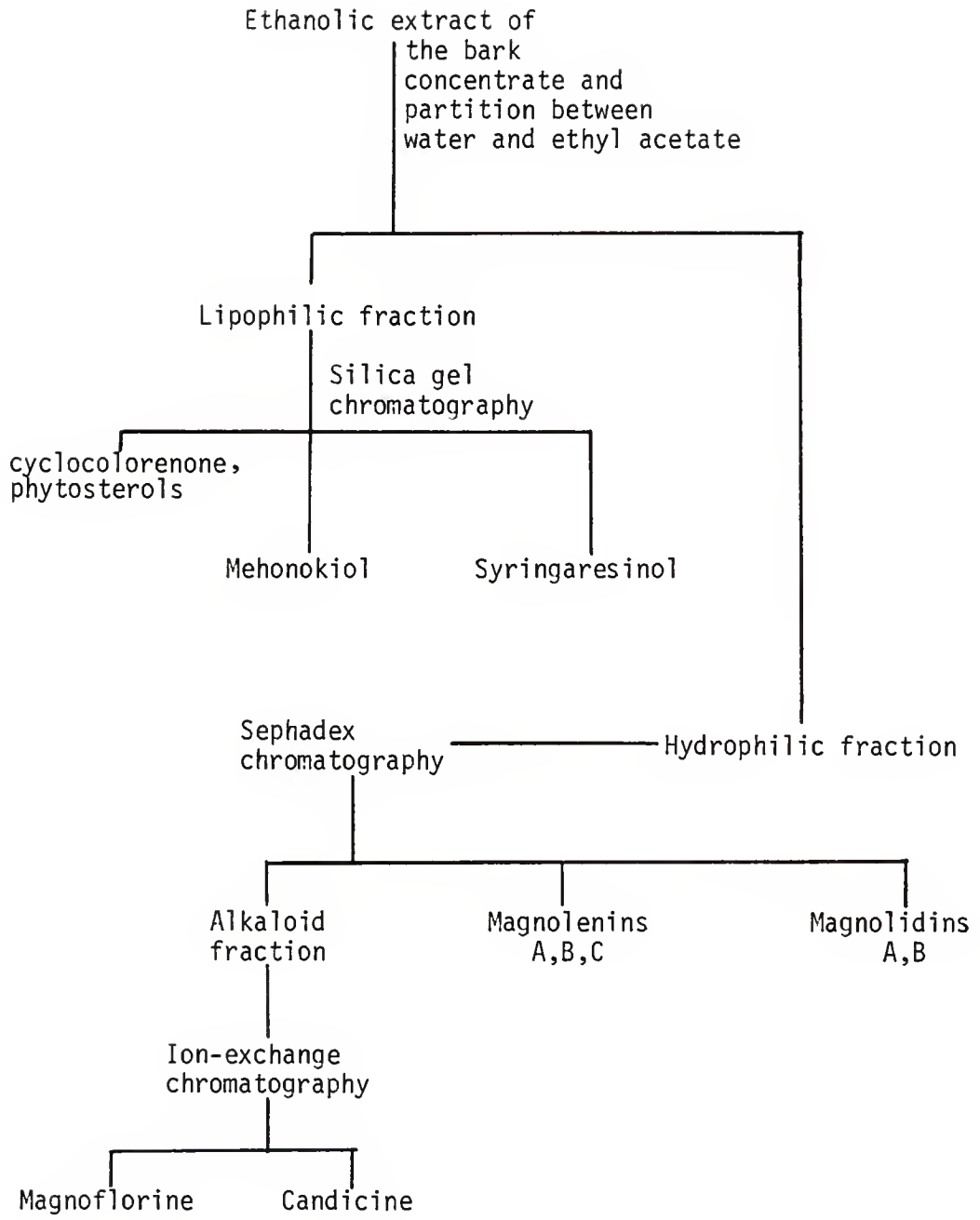


Figure 2.1 Fractionation of Magnolia grandiflora extract

samples of β-sitosterol, stigmasterol and cholesterol, all of which have been observed in the extracts of Magnolia obovata by Fujita et al.[7] Further purification of the desired compound by a repetition of the silica gel chromatography using 2.5% acetone in benzene as eluent was not satisfactory because of extensive tailing of the peak and inefficient separation from the remaining phytosterols. Substitution of Florisil as adsorbent gave, however, a sharper elution pattern and a pure sample.

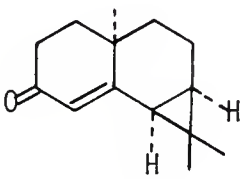
The 260 nm absorbing component was obtained as a colorless heavy oil which, however, could not be induced to crystallize. Elemental analysis and mass spectral data ($M^{+\cdot}$ 218) indicated a molecular formula of $C_{15}H_{22}O$. It showed a characteristic uv-maximum at 260 nm with log ε = 4.10. The ir spectrum showed no hydroxyl but a strong carbonyl absorption (1690 cm^{-1}) and unsaturation (1600 cm^{-1}). Its nmr spectrum gave evidence for the presence of three different types of methyl groups: 0.72 ppm, d, CH-$\underline{CH_3}$; 1.00 ppm, s, and 1.23 ppm, s, $R_2C-(CH_3)_2$; and 1.70 ppm, d, $-C=C-CH_3$; along with a considerable overlap of signals due to a CH_2- envelope. The mass spectrum, besides providing a strong molecular ion was not very helpful in giving useful structural information because of the absence of favorable, structurally meaningful fragmentation pathways. The analytical and spectral data suggested, however, that the compound might be a member of the class of sesquiterpene ketones.

A crystalline 2,4-dinitrophenylhydrazone derivative was prepared and its elemental analysis confirmed the molecular formula of the ketone.

Its uv-absorption maximum of 390 nm indicated that it was derived from an α,β-unsaturated ketone, although the λ_{max} was higher than that expected for one double bond conjugated with the keto group.[105,106] Efforts made to regenerate the ketone, possibly in purer form, by the hydrolytic exchange of the crystalline dinitrophenylhydrazone with levulinic acid and a mineral acid[107] gave a ketonic product which, however, was different from the original enone (higher R_f and higher λ_{max}). In the absence of mineral acid, no exchange took place. Thus, alteration of the structure in the presence of acid was apparent.

The λ_{max} of 260 nm observed for the ketone is intermediate in value between that of an enone (220-250 nm) and that of a dienone (280 nm).[108] The literature suggested as a model for this chromophore the cyclopropyl-conjugated enone 2.1, which was described by Büchi *et al.*, as an intermediate in their synthesis of maaliol 2.2.[109] The extent of overlap of the π-orbitals of the enone with the orbitals of the cyclopropyl ring will determine the energy of the associated electronic transition which, in turn, will be determined by the conformation of the enone and the cyclopropyl group.[110,111] In accordance with this idea, the *Magnolia* enone system might also possess a stereochemical relationship similar to that of the model compound 2.1. This was supported by the observed base-catalyzed epimerization of the enone from *Magnolia* to a product with a λ_{max} of 250 nm, apparently as a result of greater deviation from periplanarity of the enone and the cyclopropyl systems which are suspected to be present. Other evidence, also consistent with the presence of a cyclopropyl moiety, is the prominent mass

spectral fragment at m/e 175 ($M-C_3H_7$). The epimerization alluded to here, which will be discussed in more detail later, also indicated the presence of a proton either alpha to the carbonyl or at the allylic position, which can also be a ring junction.



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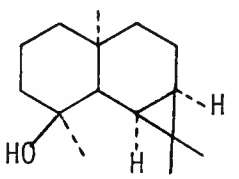

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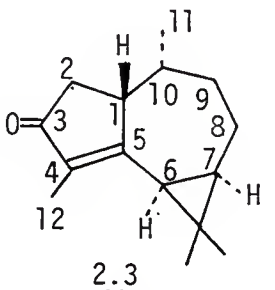


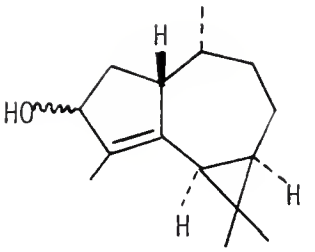
2.3

On the basis of the available information, a search of the literature was conducted which revealed that the properties of the Magnolia enone and those of its 2,4-dinitrophenylhydrazone derivative corresponded with those described for the sesquiterpene ketone, cyclocolorenone 2.3, first isolated from Pseudowintera colorata (Raoul) Dandy.[112,113] However, in the absence of an authentic sample for comparison (the compound is unstable and polymerizes on standing) and in view of much of the confusion in the literature concerning the possible presence of its epimer in the samples reported in the earlier literature, it was deemed necessary to proceed with proper structural elucidation to establish its identity.

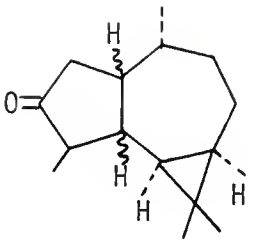
In order to establish the ring size of the enone through the infrared spectral frequency of the corresponding saturated ketone, catalytic hydrogenation by platinum in ethanol was carried out but with

no reaction. In acidic ethanol, a product was obtained which, however, showed neither a carbonyl nor a hydroxyl function.

Reduction of the enone with sodium borohydride gave the corresponding allyl alcohol 2.4 from which the starting enone could be regenerated by oxidation with manganese dioxide. This alcohol appears to correspond with cyclocolorenol 2.4 described by Corbett and Speden[113] by the action of lithium aluminum hydride on cyclocolorenone. These authors also prepared the saturated ketone 2.5 from cyclocolorenone by reduction with lithium and ammonia. This procedure was followed in the present case and the saturated ketone was obtained, except that it was a crystalline solid, mp 38-40° in contrast to Corbett and Speden's description of it as an oil. A ketone of this same structure and a melting point of 38-40° was also prepared from α-gurjunene and characterized as 4α,5α-cyclocoloranone[114] which agrees with the properties of the present ketone. However, the melting point of the dinitrophenylhydrazone of the saturated ketone from Magnolia enone differed from that of the cyclocoloranone dinitrophenylhydrazone described in the literature by either group. All these suggested that either during the reduction process or, during the formation of the 2,4-dinitrophenylhydrazone, epimerization was possible and that the melting point of the derivative might reflect the degree of epimerization. The frequency of the carbonyl band (1745 cm^{-1}) indicated, however, that the saturated ketone from Magnolia enone contained a 5-membered ring.



2.4



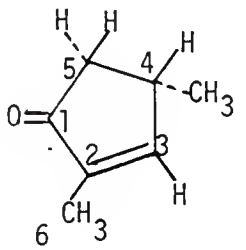
2.5

The nmr spectrum of the Magnolia enone was reported briefly earlier and a more detailed analysis will now be presented with a view to find support for its possible identity with cyclocolorenone.

The doublet at 0.72 ppm (J = 7 Hz) can be assigned to the methyl group at C-10, split by the proton at C-10, with some overlap from signals due to the methine protons of the cyclopropyl ring. The singlet at 1.00 ppm is assignable to the exo methyl and the singlet at 1.23 ppm to the endo methyl group of the gem dimethyl function. The absence of any signal due to an olefinic proton indicates that the system is fully substituted. The presence of a methyl group on the enone system is evidenced by the doublet at 1.70 ppm, and decoupling studies showed that the methyl protons are coupled to the methine proton at C-1 ($J_{1,12}$ = 1.4 Hz). The signal centered at 2.84 ppm, a doublet of doublets, represents the α-proton of C-2 coupled to the C-2β and C-1 protons with $J_{2\alpha,2\beta}$ of 18 Hz and $J_{1,2\beta}$ of 6.5 Hz. The C-1 proton appears as a multiplet at 2.94 ppm with H-2α at 2.29 ppm, $J_{1,2\alpha}$ = 2 Hz.

All of these signals show general agreement with those described by Büchi et al.,[115] for cyclocolorenone. The spectrum of the

cyclopentenone portion of the molecule may also be compared with that of 2,4-dimethylcyclopent-2-en-1-one 2.6, the pertinent parameters of which are shown in Figure 2.2.[116,117] This model compound shows ir spectral bands at 1700 and 1630 cm^{-1} for C=O and C=C, respectively,[116] in comparison with the reported values of 1690 and 1630 cm^{-1} for the respective groups of cyclocolorenone.



2.6

H-4	2.85 ppm	J_{gem}	19.5 Hz
$H\text{-}5_\beta$	2.37 ppm	$J_{4,5(cis)}$	6.5 Hz
$H\text{-}5_\alpha$	1.95 ppm	$J_{4,5(trans)}$	2.5 Hz

Figure 2.2 Nmr spectral parameters for 2,4-dimethylcyclopent-2-en-1-one

Based on the information from the model compound, additional data on cyclocolorenone were obtained by the use of double-resonance techniques. Irradiation of C-1 proton multiplet at 2.94 ppm resulted in the collapse of the C-12 allylic methyl doublet at 1.70 ppm, thus implying a long-range homoallylic coupling of these protons similar to that observed in the spectrum of α-gurjunene,[118] photosantonic acid lactone and α-santonin derivatives.[119] In α-santonin derivatives, the

homoallylic coupling is of the order of 1.3-1.4 Hz, with a critical bond angle of 115°.[118] The angle measured for a stereomodel of cyclocolorenone was found to be 115° and a coupling constant of 1.4 Hz was observed. In contrast, homoallylic coupling with the C-6 proton seems to be neglible in cyclocolorenone as might be anticipated from a 0° bond angle. This suggests that cyclocolorenone assumes a preferred conformation in which the orbital overlap of the enone and cyclopropyl systems is maximal, which occurs when the cyclopropane ring is orthogonal to the cyclopentenone ring.

The assignment of the 1.23 ppm singlet to the endo methyl of the gem-dimethyl function is based on the arguments of Streith and Ourisson[118] who state that the endo methyl group is in a relatively more deshielding region of the enone system. The carbonyl group itself must make a substantial contribution to this effect as relative deshielding is still significant in the spectrum of cyclocoloranone 2.5.

Epimerization of cyclocolorenone was observed Büchi et al.,[115] somewhat by accident, during chromatography on alumina. They tried to use this reaction in their attempted synthesis of cyclocolorenone by the epimerization of epicyclocolorenone as the last step. However, epicyclocolorenone did not produce cyclocolorenone under conditions which produced the reverse reaction. Corbett and Young[120] use boiling ethanolic potassium hydroxide for epimerization. The enone from Magnolia was subjected to epimerization both by passage through by an alumina column as well as by boiling in alcoholic potassium hydroxide. Although the

product showed the expected shift in λ_{max} toward 250 nm, thus indicating epimerization had taken place, its nmr spectrum showed the reaction to have proceeded only to the extent of 50-75%. (The mixtures show two pairs of gem-dimethyl signals, the ratios of which vary with the extent of epimerization.) The mixture was inseparable by tlc or by gas chromatography. Also, the reaction with alcoholic base was rather drastic and a significant extent of degradation accompanied epimerization. The mixture resulting from the best epimerization reaction of the Magnolia enone was converted to the 2,4-dinitrophenylhydrazone, which was recrystallized to a constant melting point. The melting point and spectral data of this product were consistent with those described for the epicyclocolorenone,[115,120] although this derivative was also unresolvable chromatographically from the dinitrophenylhydrazone of cyclocolorenone. Thus, the situation with the epimerization is far from clear, either in the present work or in the descriptions that appear in the literature. Another reason to suspect this is that the melting point of the cyclocoloranone 2,4-dinitrophenylhydrazone varies over a range, again attributable to an undetermined degree of epimerization.

The discrepancies observed, reflecting the proclivity of the molecule to undergo epimerization, might readily have been anticipated. It was felt that, given the analytical and nmr data, and the unusual chromophore, the identity of this compound is no less certain. As final proof, however, copies of the ir and nmr spectra of synthetic cyclocolorenone, obtained from Dr. D. Caine,[121,122] and of the Magnolia enone are compared in Figures 2.3 and 2.4 (an impurity appearing on the carbonyl band diminishes as the sample is purified).

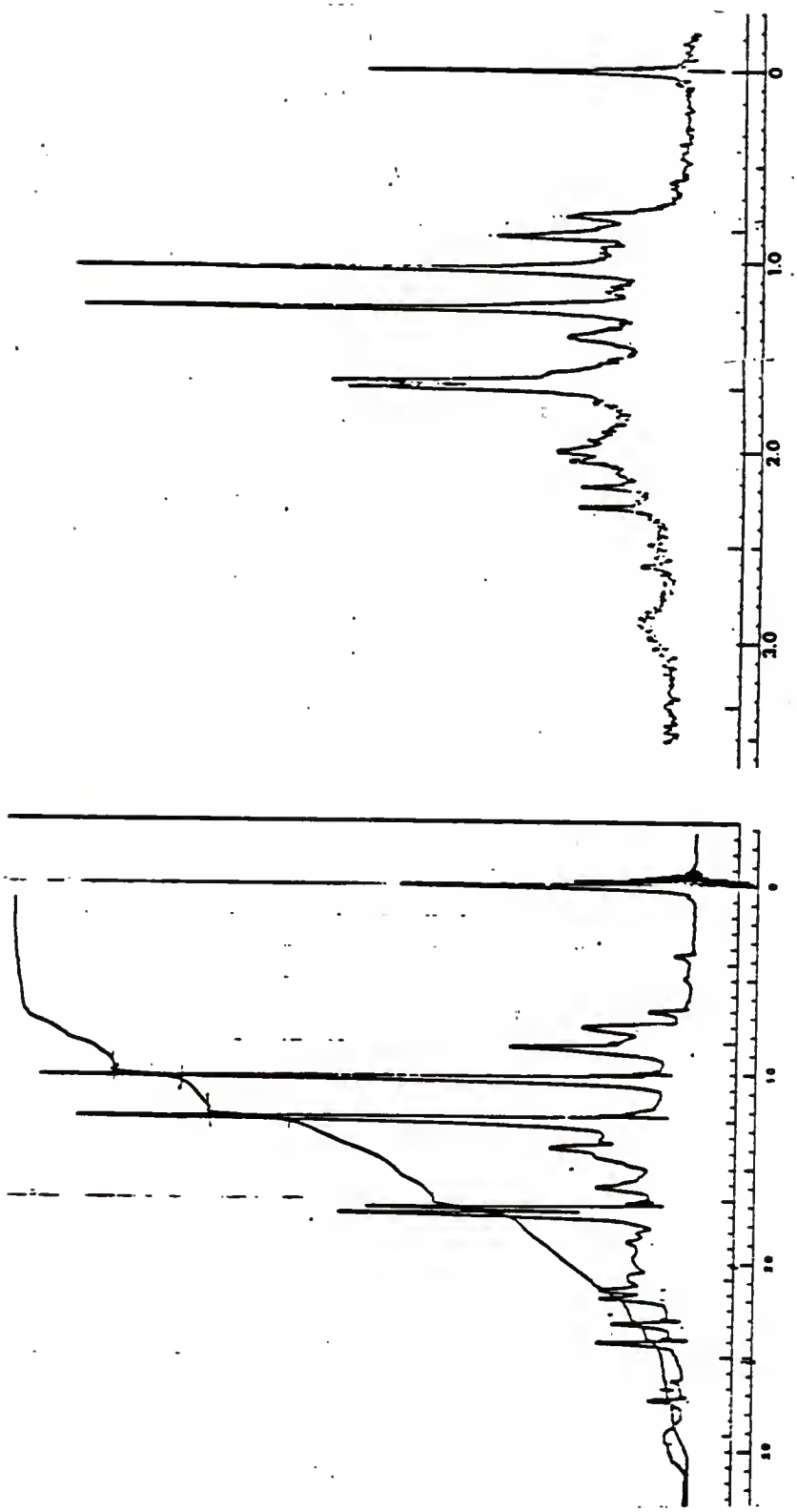


Figure 2.3 Comparison of nmr spectra of (a) Magnolia enone and (b) synthetic cyclocolorenone

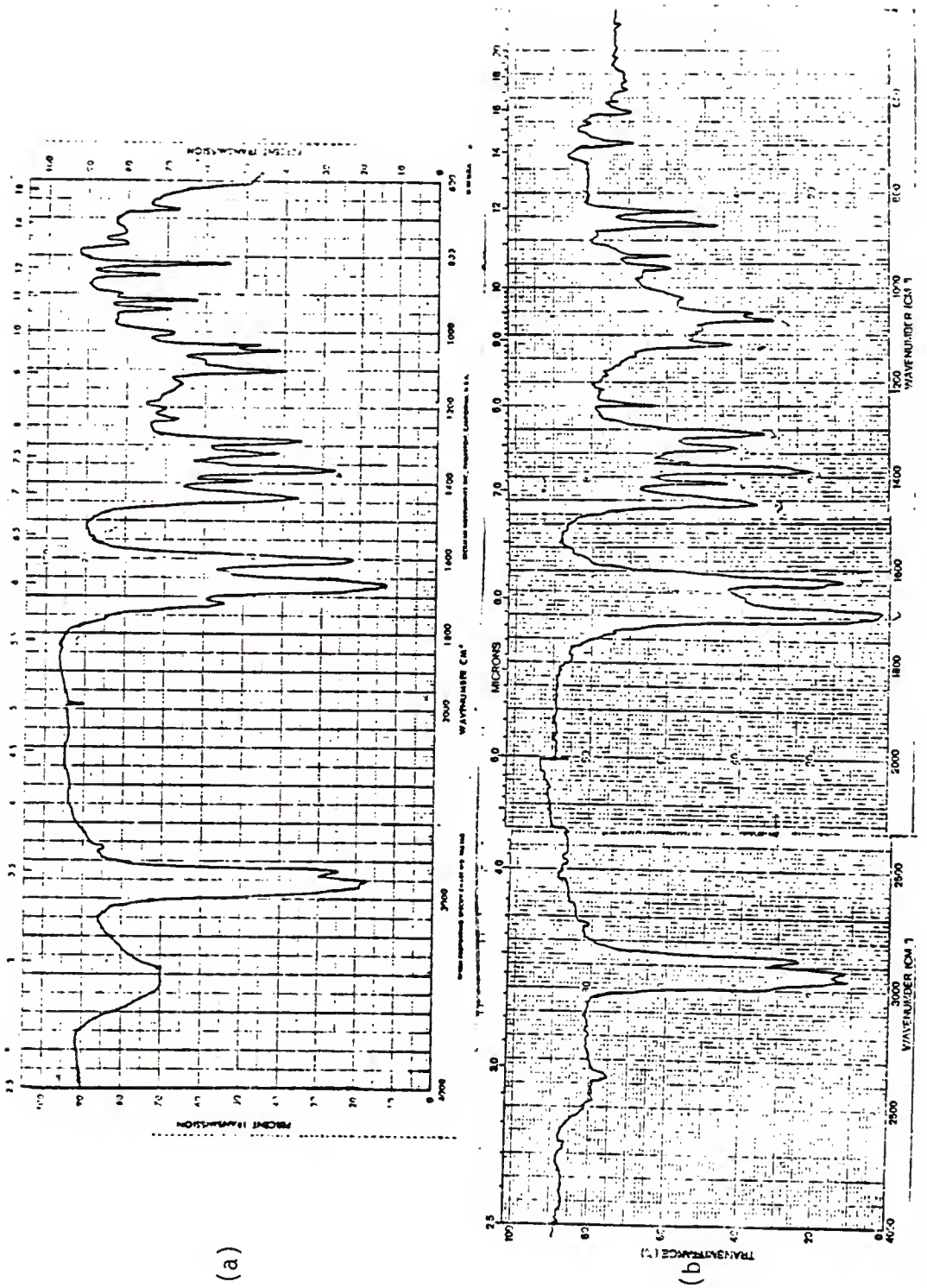


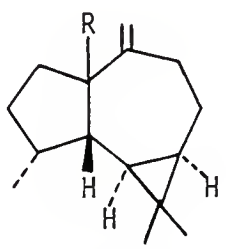
Figure 2.4 Comparison of ir spectra of (a) Magnolia enone and (b) synthetic cyclocolorenone

Cyclocolorenone was first isolated from Pseudowintera colorata, a plant which the authors ascribe to Magnoliaceae, although it is usually assigned to Winteraceae.[123-126] These two families are considered by many authors to be among the most primitive of the Angiospermae. Recently, cyclocolorenone has been isolated from an even more primitive plant, the liverwort, Plagiochila acanthophylla, subspecies Japonica (Hepaticae).[127] In addition to these, the compound was found to be present in Boronia ledifolia Gay (Rubiaceae)[128] and in the goldenrod, Solidago canadensis (Compositae).[129]

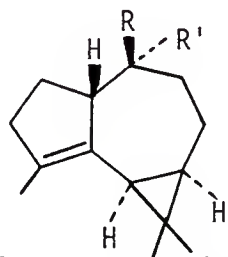
Although the basic structure of cyclocolorenone was determined by the authors who isolated it originally, its stereochemistry was determined largely by Büchi et al.,[115] through their synthesis of epicyclocolorenone, and from its relationship to α-gurjunene.[114,118] Cyclocolorenone itself was finally synthesized by Ingwalson and Caine starting from (-)-maalione.[121,122]

Cyclocolorene is a member of what has recently been described as ". . . the rare class of aromadendrane sesquiterpenes."[130] The class appears to be neither so rare nor so restricted in its membership as has been previously thought, numbering at present some fifteen compounds, some of which are widely distributed. Nearly all of the members contain the cyclopropyl ring in the 6S,7S-configuration of (-)-cyclorocolorenone. The fusion of the 5- and 7-membered rings can be either cis or trans, aromadendrene 2.7 itself being 1α,5β while alloaromadendrene 2.8 is 1β,5β;[131,132] α-gurjunene 2.9[118,133-135] and

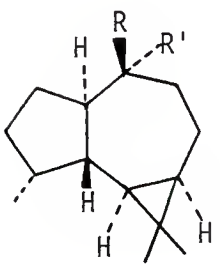
β-spathulene 2.10[136] represent the prototypes with a 4,5-double bond and 1β hydrogen: cyclocolorenone is of this type. This relationship between cyclocolorenone and α-gurjunene is accentuated further by the fact that the two occur together in Pseudowintera colorata,[137] and there has been speculation that cyclocolorenone might have been an artifact arising from α-gurjunene on long storage through air/photo-oxidation.[118] A number of alcohols derived from either aromadendrane (e.g., globulol 2.11[131] and spathulenol 2.12)[138] or alloaromadendrene (e.g., ledol 2.13,[118] viridiflorol 2.14[118]) have been described. An unusual isonitrile, axisonitrile II 2.15[139] and derivatives 2.16 and 2.17[140] have been isolated from the sponge, Axinella canabina (Porifera).



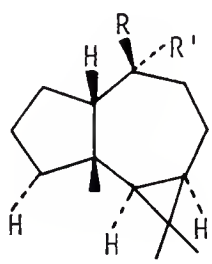
2.7, R = αH
2.8, R = βH



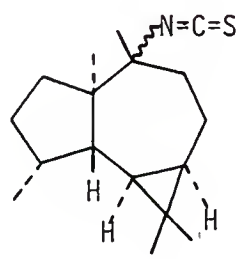
2.9, R = H, R' = CH_3
2.10, R = R = $>CH_2$



2.11, R = CH_3, R' = OH
2.12, R = R' = CH_2



2.13, R = CH_3, R' = OH
2.14, R = OH, R' = CH_3



2.15, R = -N=C
2.16, R = -N=C=S
2.17, R = -NHCHO

Antibiotic Principle of the Leaves

The extract of yellowed leaves of *Magnolia grandiflora* was found to possess antibiotic activity when assayed with *Bacillus subtilis* using the disk-plate method. Partitions between ethyl acetate and water at pH 9 and pH 2 indicated that the antibiotic principle is a neutral molecule, readily extractable in this solvent.

The extract was therefore partitioned between ethyl acetate and dilute aqueous ammonium hydroxide (pH 10) to remove phenolic materials, and the combined solvent phases chromatographed on silica gel-cellulose. The activity was found in the 5% acetone/benzene eluate, associated wtih a component of R_f 0.38 (5% acetone/benzene), which turned bright red when sprayed with sulfuric acid reagent and heated. The component was also active in the antibiotic assay.

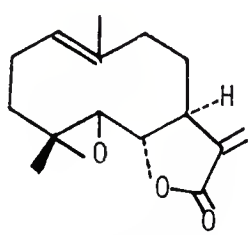
Although the antibiotic was also found to be present in fresh green leaves in approximately the same amounts, purification was more difficult because of the chlorophyll, which appeared in the same chromatographic fractions. Despite the fact that the chlorophyll was removable by chromatography on Florisil, it was found to be more convenient to use the yellow leaves.

With this method of purification, the extract of 500 g of leaves afforded 41 g of material on concentration, with a minimum inhibitory concentration (MIC) of 400 g/ml. Upon partition, the solvent phase yielded 5 g, MIC 55 g/ml. Chromatography on silica gel-cellulose yielded an antibiotic-containing fraction, 672 mg of an oily liquid, which was extracted with petroleum ether, the extract concentrated to

an oil and triturated with ethanol to give a solid. Crystallization from ether gave large, colorless crystals, 210 mg, mp 112-114°C, and MIC 3 μg/ml.

The mass spectrum (M^+ 248) and elemental analysis are consistent with the molecular formula $C_{15}H_{20}O_3$. The ir spectrum (KBr) showed an ester carbonyl (1738 cm^{-1}), C=C (1655 cm^{-1}), and a possible terminal methylene group (890 cm^{-1}). The nmr spectrum confirmed the presence of the latter through signals at 5.72 and 6.15 ppm, 2d, J = 3.6 Hz, and showed the following peaks: 5.25 ppm, m, 1H, vinylic; 3.98 ppm, 1H, t, J = 18.4 Hz, $R_2\underline{CH}OR$; 2.87 ppm, 1H, d, J = 18.4 Hz, epoxide methine; 1.85-2.40 ppm, 8H, CH_2 envelope: 1.70, singlet with fine structure, allylic methyl group; and 1.23 ppm, 1H, s, methine. The uv spectrum showed only end absorption.

The melting point and spectral data are in agreement with those reported for parthenolide 2.16 by Govindachari et al.[4] and a comparison of their nmr spectrum with that of the Magnolia antibiotic is shown in Figure 2.5.



2.16

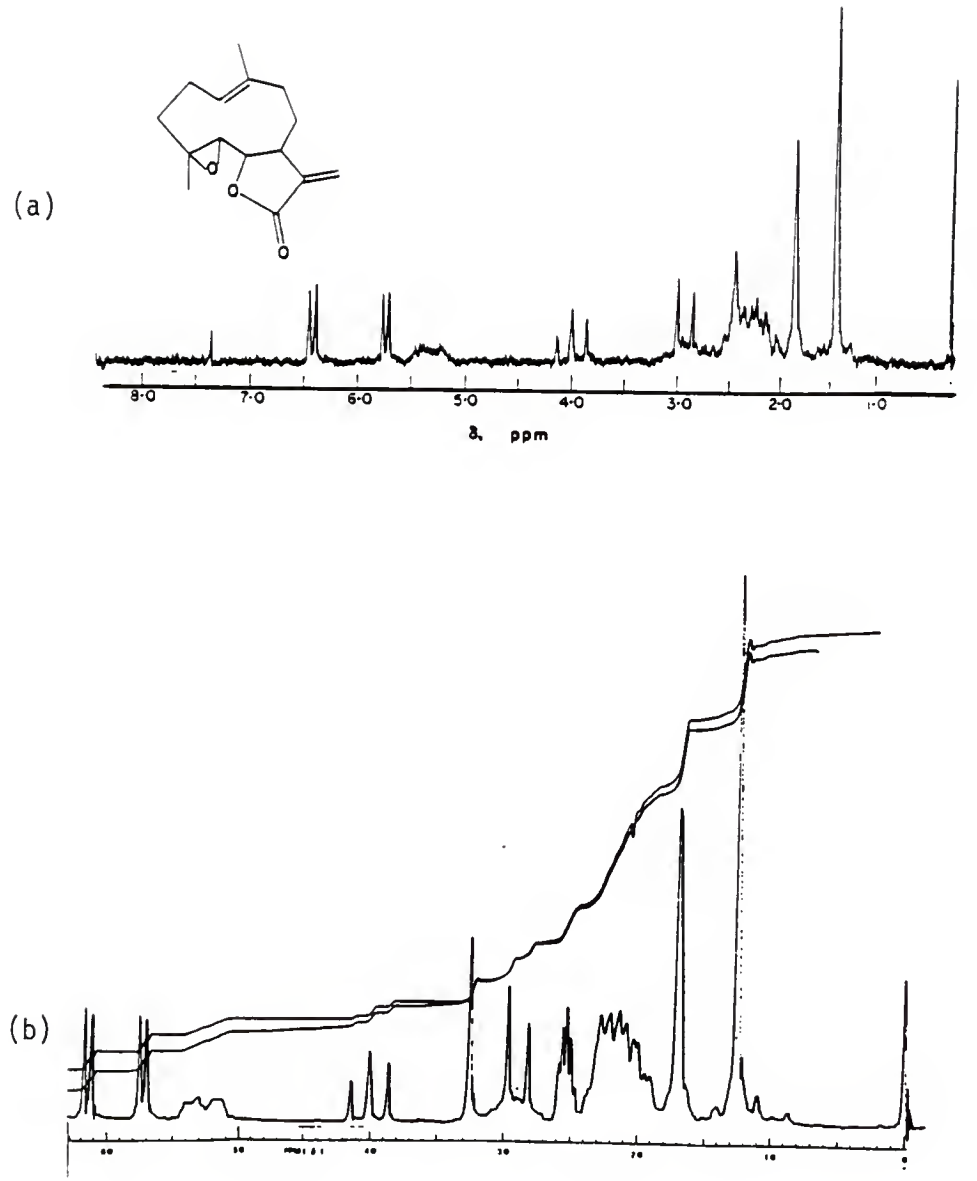


Figure 2.5 Comparison of nmr spectra of (a) parthenolide and (b) Magnolia antibiotic (D_6DMSO)

The melting point and ir spectra are in agreement with the published data of Govindachari et al.,[4] though the carbonyl band is distorted to a lower frequency, probably a result of having been determined in the solid state (KBr).

This compound was originally isolated from Chrysanthemum parthenium (l.) Bernh.[141,142] and subsequently from Ambrosia dumosa Gray,[143] and Ambrosia confertiflora DC.,[144] all Compositae. Indeed, the Compositae, and particularly Ambrosia and their relatives, elaborate a large variety of sesquiterpene lactones.[144] Such compounds are much less prevalent in other families.

Within the Magnoliaceae, in addition to M. grandiflora,[6] parthenolide has been isolated from Michelia campaca L. by Govindachari et al.,[4] who revised the original structure proposed by Herout et al.[141] and Soucek et al,[142] and from Michelia lanuginosa L.[145] The antibiotic activity of this compound has not been previously described.

The stereochemistry of parthenolide was determined by Bawdekar et al.,[146] who correlated it with the known stereochemistry of costunolide.

Parthenolide and costunolide are the only sesquiterpene lactones reported from Magnolia. Interestingly, a number of related costunolide derivatives are known from the magnoliaceous tree, Liriodendron tulipifera.[147,148] These, like parthenolide, have antitumor activity.[147]

Experimental

Extraction of Magnolia bark: Cyclocolorenone 2.3

Ground bark (41 g) of Magnolia grandiflora, collected in Gainesville, Florida, was extracted with ethanol (10 ℓ) at 25° C for two

days. Three such extracts were combined, concentrated at 20 mm pressure to a syrup which was partitioned between water and ethyl acetate (2 ℓ each). Concentration of the solvent layer gave a heavy oil (120 g).

An aliquot of the oil (20 g) was chromatographed on silica gel cellulose (500 g) in benzene. Fractions (10-12 ml) were collected and tested by absorbance (260 nm) and thin layer chromatography. The benzene eluate contained besides carotenoid and other highly lipid-soluble components, the phenylpropanoid component of which is described in Chapter 3. The column was then eluted with 5% acetone in benzene which eluted cyclocolorenone, recognizable by its tlc behavior and the uv-maxima at 260 nm, as well as phytosterols which moved in tlc at slightly lower R_f and produced a purplish-brown color when sprayed with 1% sulfuric acid/acetic acid and heated gently. The mixture of the cyclocolorenone and phytosterols was dissolved in methanol (10 ml) and kept at 5°C overnight. The precipitate was filtered off and set aside for gas-chromatographic study.

The filtrate and wash were concentrated to an oil, dissolved in benzene (20 ml) and applied to a column of Florisil (100 g) in the same solvent. The fractions which contained the ketonic component were combined and concentrated to yield a colorless, heavy oil; yield, 0.5% of the bark by weight; homogeneous in tlc and gc (T 175°, retention time on 3% OV-17, 6 ft.; 5 min.; on 3% OV-1: 3 min.); uv: λ_{max} 264 nm; log ε 4.12 (lit. 264/4.12)[113] mass spectrum: m/e 218 (M^+ 100%), 203, 175, 162, 161, 149, 147, 134, 133, 119, 105, 93 and 91.

Analysis of the Phytosterol Fraction

The column consisted of 3% OV-17 on Gaschrom Q, 6 ft., programmed from 150-275°C at 10° per minute. The mixture showed components with retention characteristics similar to those of cholesterol (Aldrich), lanosterol (Mann Research) and β-sitosterol (Fisher). Under isothermal conditions at 250°C, the retention times are as follows:

cholesterol	13.5 min.
lanosterol peak 1	19.0 min.
peak 2	20.5 min.
β-sitosterol peak 1	22.0 min.
peak 2	24.5 min.
peak 3	27.0 min.

Each of these peaks was homogeneous with the corresponding peaks obtained from the mixture.

Cyclocolorenone 2,4-dinitrophenylhydrazone

A solution of the 2.3 (0.4 g) in methanol (20 ml) containing 2,4-dinitrophenylhydrazine (0.4 g) and 6N HCl (0.5 ml) was heated at 50°C for 15 minutes. After cooling for one hour, the dark red solid was filtered and washed with cold methanol. It was recrystallized from ethyl acetate; yield, 0.5 g (70%), mp 217.5°C (lit. 218°);[113] uv λ_{max} 400 nm, log 4.51 (lit. 404, log 4.40);[113] mass spectrum: m/e 398, 356, 105, 91, 77, 55, 43 and 41.

Anal. calc. for $C_{21}H_{26}O_4N_4 \cdot H_2O$: C, 60.56, H, 6.78; N, 13.45. Found: C, 60.62; H, 7.31; N, 13.19.

Reduction of Cyclocolorenone to Cyclocolorenol 2.4

A solution of 2.3 (0.1 g) in absolute ethanol (5 ml) was treated with sodium borohydride (0.05 g) and the mixture stirred for 30 minutes.

After five minutes at 50°C, it was cooled, neutralized with 1N HCl and concentrated to a small volume. Extraction with ether, drying of the extract and concentration gave 2.4 a colorless oil; yield, 0.07 g; uv: no absorption above 220 nm; ir: 3200-3600 cm^{-1}.

The sample in hexane (10 ml) was stirred at 25°C with activated manganese dioxide (Baker and Adamson) for two hours. Filtration and concentration of the filtrate gave an oil, identical with 2.3 in tlc, and uv spectra.

Reduction of Cyclocolorenone to Cyclocoloranone 2.5

The procedure was an adaptation of the one described by Corbett and Speden:[113] in a flask equipped with an acetone dry-ice condenser and containing anhydrous ammonia (500 ml) was dissolved lithium (1.89 g). The ketone (5 g) in anhydrous ether (100 ml) was added dropwise with stirring over a period of one hour. After another 30 minutes, ammonium chloride (1.5 g) was added in small portions and the ammonia allowed to evaporate overnight. The solid mass was stirred with ether, filtered, the filtrate washed with salt solution, dried and concentrated to dryness. It was dissolved in 1:1 benzene/hexane and chromatographed on alumina (100 g) in the same solvent. Fractions from the major band on concentration gave 2.5, a crystalline solid, recrystallized from hexane; mp 38-40°; yield 1 g (20%).

Anal. calc. for $C_{15}H_{24}O \cdot H_2O$: C, 75.69; H, 10.92. Found: C, 76.48; H, 10.79; ir: 1745 cm^{-1}; nmr (ppm): 1.7-2.3, 6H; 1.26, s, 3H; 1.05, s, 3H; 1.11, d, 3H, J = 8.5 Hz; 0.90, d, 3H, J = 7.8 Hz and 0.75-1.60, m, 5H; mass specrum; m/e 220 (M^+), 177, 149, 136, 135, 122, 109.

Cyclocoloranone 2,4-dinitrophenylhydrazone

This was prepared as described under the cyclocolorenone dinitrophenylhydrazone. It is a light orange crystalline solid, mp 207-210° from ethyl acetate; uv: λ_{max} 360 nm, log 4.33.

Epicyclocolorenone

A 200 mg sample of 2.3 was chromatographed on a 50 g column of neutral alumina (Woelm, activity grade 1) in benzene. Elution with 2% acetone/benzene gave a product, identical with 2.3 but with a λ_{max} of 255 nm instead of 264 nm. The nmr spectrum showed duplicate signals for the geminal methyls at 1.25, 1.23 ppm, and 1.04, 1.01 ppm, indicating a mixture containing 70% of 2.3. Repetition of this procedure with the same sample gave λ_{max} 250 nm, with nmr indicating 40% 2.3, yield 150 mg.

According to the procedure of Corbett and Young,[120] 2.3 (200 mg) was heated under reflux in 50 ml of ethanolic KOH (0.5 N) for two hours, cooled, neutralized (H_2SO_4), and reduced in volume to 10 ml. Unlike these authors, chilling overnight did not afford a solid product. The solution was diluted to 50 ml with water and extracted three times with ether. The ether was dried (Na_2SO_4) and concentrated to give an oil, 190 mg, which could not be induced to crystallize. The product was homogeneous (tlc) with 2.3, λ_{max} 255 nm. The product was isolated by chromatography on silica gel (5% acetone in benzene) to afford 70 mg of a ketone, ir 1610 cm^{-1}, homogeneous with 2.3 on gc under the conditions cited for cyclocolorenone, nmr indicated 40% epimerization.

Epicyclocolorenone 2,4-dinitrophenylhydrazone

The product of alumina-induced epimerization, 150 mg, was converted to the DNP derivative as described for 2.3. Four recrystallizations from ethyl acetate gave a product of constant mp, 188-189°C (lit. 189),[120] homogeneous with 2.3-DNP on tlc, uv 400 nm, log 4.48/chloroform (lit. 397.5 nm/4.49).[120]

Parthenolide 2.16

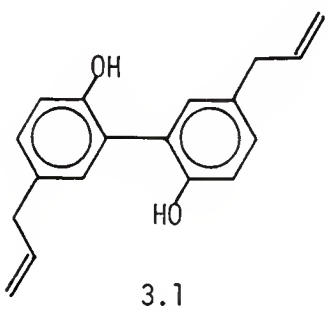
Yellowed leaves of M. grandiflora (500 g) were cut into small pieces and extracted with ethanol for one day at 25°C. Three such extracts were combined, concentrated to a syrup which was then partitioned between 0.1 N ammonium hydroxide and ethyl acetate (250 ml each). The solvent layer was concentrated to dryness, the residue dissolved in benzene (10 ml) and applied to a column of Florisil (50 g) in the same solvent. Elution with 5% acetone in benzene gave the antibiotic fraction which was recovered by concentration. The resulting oil was stirred with hexane, filtered and the solid washed with hexane. The filtrate was again concentrated to an oil which was triturated with ethanol (1 ml). The crystalline solid was filtered and recrystallized from ether-hexane; yield, 0.5 g; mp 112-4° (lit. 115°C)[145]; ir: 1730 and 1655 cm^{-1}; mass spectrum: m/e 248 (M^{+}), 233, 230, 190 and 43.

Anal. calc. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: 72.33: H, 8.17.

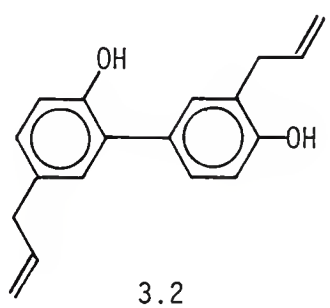
CHAPTER 3
PHENYLPROPANOIDS OF Magnolia grandiflora L.

Bis-allylphenol from the Bark

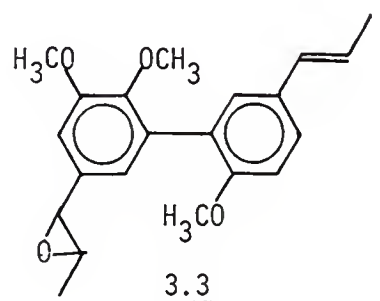
The distribution of diallylbiphenol derivatives in Magnolia spp. was discussed in Chapter 1. So far, three members of this group have been isolated from Magnolia spp., magnolol 3.1,[12,53-54] honokiol 3.2[12,54-57] and acuminatin 3.3.[9,53] A search for these or related compounds in Magnolia grandiflora was made but with negative results.[55] In the present study, a new member of this group, a derivative of honokiol has been obtained from M. grandiflora and its isolation and elucidation of its structure from the subject of this chapter.



3.1



3.2



3.3

The lipophilic fraction of the ethanolic extract of the bark of Magnolia grandiflora when subjected to chromatography on silica gel-cellulose (1:1) yielded a component with a characteristic uv absorption with a λ_{max} of 290 nm. The phenolic nature of the compound was demonstrated by the shift in λ_{max} to 320 nm in the presence of

base. However, the compound was still contaminated with other non-acidic components and was too lipophilic to be extracted into aqueous base from solvents such as chloroform, benzene or ether, which might explain why the previous workers did not recognize its presence. It was, however, possible to separate this phenolic component from the neutral compounds by partition between hexane and aqueous methanolic (1:1) base. Further chromatography on silica gel gave the pure phenolic component in a yield of 0.005-0.01% which was homogeneous by thin-layer chromatography in two different systems and by gas chromatography. With the help of this solvent-partition scheme and gas chromatography, the extract of the wood of M. grandiflora was examined for the presence of this phenolic component but it was found to be absent.

The phenolic component is a colorless oil with the molecular formula $C_{19}H_{20}O_2$ on the basis of elemental analysis and mass spectrum (M+ 280). Its uv spectrum showed maxima at 255 and 290 nm with log ε of 4.10 and 3.8, respectively, which shifted to 320 nm on the addition of base. Its ir spectrum--3200-3600 cm^{-1} (phenolic OH), 1620 cm^{-1} (aromatic, 985, 905 cm^{-1} ($CH=CH_2$)--indicated the presence of phenolic and olefinic functions. The nmr spectrum--6.65-7.22 ppm, m, 6 aromatic H; 5.55-6.32 ppm, m, 2 $CH_2\underline{CH}=CH_2$; 4.92 ppm, m, and 5.11 ppm, m, 2 $CH_2CH=\underline{CH_2}$; 4.86 ppm, s, exchangeable, $ArO\underline{H}$; 3.85 ppm, $ArO\underline{CH_3}$, 3.28, 3.40 ppm, overlapping doublets, J = 6 Hz, 2 $Ar\underline{CH_2}CH=CH_2$--clearly indicated that there are two non-equivalent allyl groups on aromatic rings comprising 1,2,4 substitution patterns.

The allyl groups were readily reducible to afford a tetrahydro compound. Elemental analysis and the mass spectrum (M^+ 284) were consistent with the molecular formula $C_{19}H_{24}O_2$. The nmr spectrum showed two non-equivalent benzylic n-propyl groups: 2.40 and 2.62 ppm, overlapping triplets, J = 5.2 Hz, 2 $Ar\underline{CH_2}CH_2CH_3$; 1.63 and 1.67 ppm, overlapping sextets, J = 8 Hz 2 $ArCH_2\underline{CH_2}CH_3$, 0.94 and 0.97 ppm, overlapping triplets, J = 8 Hz, 2 $ArCH_2CH_2\underline{CH_3}$.

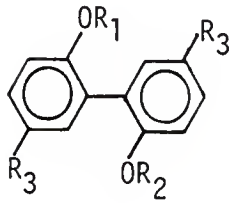
Acetylation of the phenolic component gave a monoacetate $C_{21}H_{22}O_3$ (M^+ 322). The ir spectrum of the acetate (1755 cm^{-1}) and the nmr signal (2.02 ppm, s, 3H) showed that the compound was a phenolic acetate.

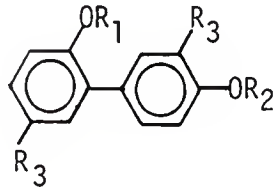
Methylation provided a monomethyl ether $C_{20}H_{22}O_2$ (M^+ 294) which showed no hydroxyl signal in its ir spectrum and two singlets 3.68 and 3.71 ppm, each representing a methoxyl group, in its nmr spectrum.

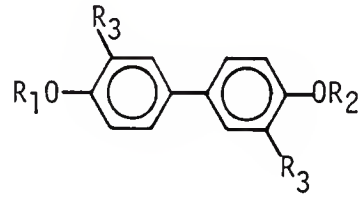
The preceding data suggested the possibility that the lipophilic phenolic component of Magnolia might be one of the monomethyl ethers of the diallylbiphenols: magnolol 3.1, honokiol 3.2, or 3,3'-diallyl-4,4'-dihydroxy-biphenyl 3.4 (which has not yet been isolated from a natural source). To confirm this possibility and to determine which of the three substitution patterns corresponds to the phenol in question, the respective di-O-methyl tetrahydro-derivatives 3.5, 3.6 and 3.7, respectively, of the three were synthesized for comparison with the methylated, reduced phenol from Magnolia.

For the synthesis of the dipropyl biphenols 3.8, 3.9 and 3.10, there are several methods based on oxidative coupling of phenols available using ferric ion,[149] hydrogen peroxide,[150] hydrogen peroxide and

ferrous sulfate,[151] and enzymic catalysis.[152] However, not only are the yields in thse reactions low (10%) but with multiplicity of sites for coupling, unequivocal assignment of structures becomes difficult, esepcially when unsymmetrical coupling is involved. Hence, the Ullmann coupling procedure employed by Fujita et al.[12] was selected as a better synthetic route because of the greater certainty of the course of reaction and relatively higher yields. The necessary iodo compounds are accessible from the commercially available p-propyl phenol 3.11 and the o-allyl phenol 3.12.







3.1, $R_1 = R_2 = H$, $R_3 = CH_2CH{=}CH_2$

3.2, $R_1 = R_2 = H$, $R_3 = CH_2CH{=}CH_2$

3.4, $R_1 = R_2 = H$, $R_3 = CH_2CH{=}CH_2$

3.5, $R_1 = R_2 = CH_3$, $R_3 = CH_2CH_2CH_3$

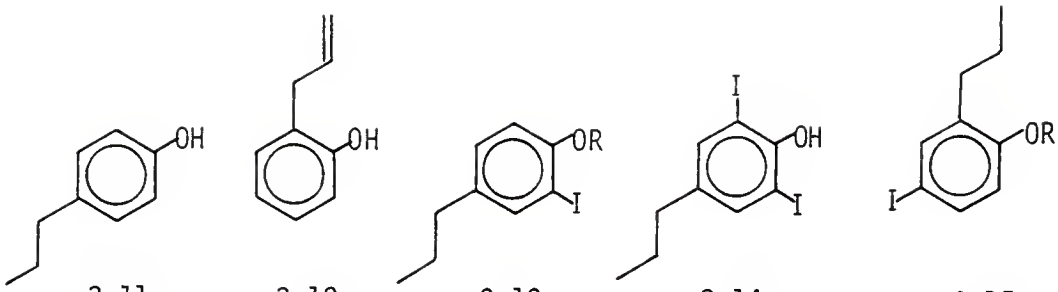
3.6, $R_1 = R_2 = CH_3$, $R_3 = CH_2CH_2CH_3$

3.7, $R_1 = R_2 = CH_3$, $R_3 = CH_2CH_2CH_3$

3.8, $R_1 = R_2 = H$, $R_3 = CH_2\ CH_2CH_3$

3.9, $R_1 = R_2 = H$, $R_3 = CH_2CH_2CH_3$

3.10, $R_1 = R_2 = H$, $R_3 = CH_2CH_2CH_3$



3.11 3.12 3.13 3.14 3.15

3.13, R = H
3.16, R = CH_3

3.15, R = H
3.17, R = CH_3

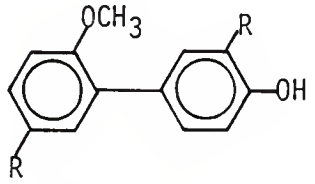
For the preparation of the iodo compound 3.13, iodination of 3.11 in aqueous base was employed. The yield of the monoiodo compound 3.13 did not go beyond 50%, due to competing formation of the diiodo compound 3.14, as determined by gas chromatography. Because of the limited yield and the difficulty of large-scale separation of 3.13 and 3.14, an alternative procedure described by Hata and Satō,[153] using iodine and mercurous oxide, was found to be preferable. The monoiodo compounds 3.14 and 3.15 were readily prepared by this procedure with no competing iodination of the iodophenols, and converted to their respective methyl ethers 3.16 and 3.17, suitable for the coupling reaction.

The method adopted for the Ullmann reaction is essentially that of Fujita et al.[153] using copper powder activated by the procedure of Kleidner,[154] and heating without solvent at 260°C. Maintaining the reaction at this temperature for six hours after completion of addition of the copper was found to be preferable to increasing the temperature as recommended by Fujita et al.[12]

Under these conditions, dimethyl tetrahydromagnolol 3.5 was obtained in a 30% yield from 3.16. Coupling of 3.17 provided

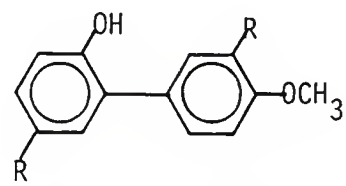
3,3'-di-n-propyl-4,4'-biphenol dimethylether 3.17 in a 60% yield as a colorless, crystalline solid. Crossed coupling of 3.16 and 3.17 in equimolar proportions yielded a mixture of the three expected products, 3.5, 3.7, and dimethyl tetrahydrohonokiol 3.6 in a ratio of 1:1:2 as determined by gas chromatography. The dimethyl ethers were converted to the corresponding biphenols 3.8, 3.9, and 3.10 by hydrolysis with HI.

The methylated, hydrogenated phenolic component from Magnolia was found to be identical with dimethyl tetrahydrohonokiol 3.6 by spectral, thin-layer, and gas-chromatographic comparison. Since the natural product is a monomethyl ether, it still remained to determine which of the two monomethyl ethers of honokiol, 3.18 or 3.19, actually represents the correct structure for the phenolic component from Magnolia.



3.18, R = $CH_2CH{=}CH_2$

3.20, R = $CH_2CH_2CH_3$



3.19, R = $CH_2CH{=}CH_2$

3.21, R = $CH_2CH_2CH_3$

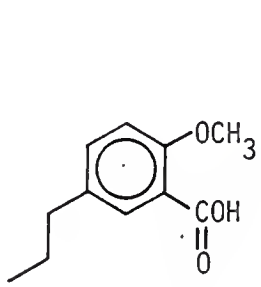
A number of alternatives was considered for providing an unequivocal choice between 3.18 and 3.19. For example, if the natural product had the structure 3.18, an acid-catalyzed cyclization to a furan or a chroman might be possible, or oxidation of the allyl functions to carboxyls can result in the formation of a salicylic acid derivative. Either the spectral properties of the cyclic ether or the

spectral and complexing properties of the salicylic acid derivative would permit a choice to be made. However, if 3.19 were the correct structure, both of these would be negative and it is not prudent to rely on negative data. Since the supply of the natural product was also very low, the selected method must be based more on a more readily available synthetic compound such as 3.8. If the isomeric monomethyl ethers 3.20 and 3.21 of tetrahydrohonokiol were synthesized and their respective structures established, identity of the tetrahydro derivative of the natural product with one of these isomers of known structure would provide the structure for the natural product.

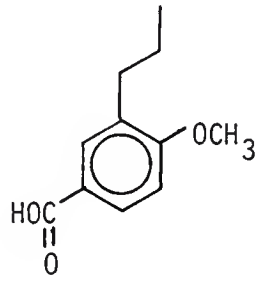
Accordingly, with the expectation that possible influence of the n-propyl group on the ortho-methoxyl of 3.6 might result in selective demethylation, reaction of 3.6 with anhydrous aluminum chloride was studied. The reaction, however, yielded both monomethyl ethers 3.20 and 3.21 in equal amounts, along with the fully demethylated 3.9. Alternatively, complete demethylation of 3.6 to 3.9 and careful partial methylation gave the mixture of 3.20 and 3.21 in a better yield. Based on the thin-layer chromatographic R_f values, they were designated as ether-A (higher R_f) and ether-B (lower R_f). Of these, A was found to be identical with the tetrahydro derivative of the natural product, and it remained to associate the ether-A with 3.20 or 3.21 to establish the choice between 3.20 and 3.21.

For a definitive assignment of the structures of the ethers A and B (3.20 and 3.21), a method based on degradation of the aromatic ring which carries the phenolic hydroxyl to yield an n-propyl anisic

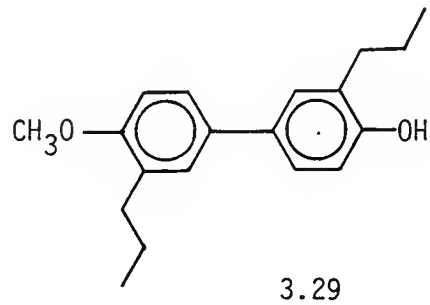
acid, followed by establishment of its identity by synthesis was found to be the most conclusive. Thus, 3.20 and 3.21 under conditions of such degradation will yield the acids 3.22 and 3.23, respectively.



3.22



3.23

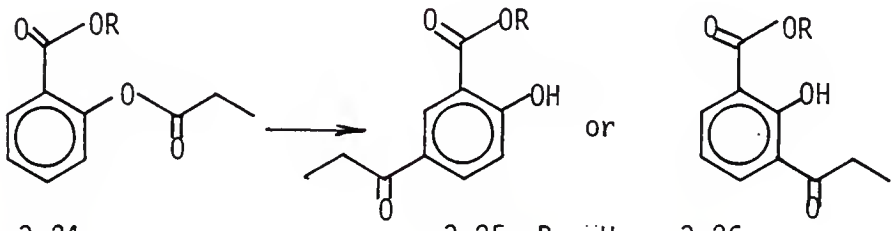


3.29

For the degradation of the phenolic ring, ozonolytic cleavage was selected. Although no difference was noted in the rate of degradation by ozone of phenol and anisole, used as model compounds, in a neutral medium, phenol was degraded rapidly in a basic medium while anisole was relatively unaffected. The monomethyl ether 3.29 prepared from 3.10, upon ozonolysis at pH 9, followed by a brief treatment with potassium permanganate, readily yielded 3.23 whose analytical and spectral data indicated that it was an n-propyl anisic acid. In a similar manner, the ether-A (3.21) gave the acid 3.23 and the ether-B (3.20), the acid 3.22. The identity of these two acids remained to be established through synthesis.

A search of the literature revealed that although related compounds were described, neither of the acids 3.22 of 3.23 are known. It was proposed to synthesize these acids by Friedel-Crafts acylation of the appropriate hydroxybenzoic acids, followed by Clemmensen reduction of

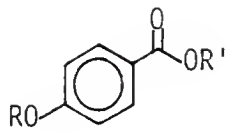
the keto groups. With regard to acid 3.22, 5-propionyl salicylic acid 3.25 has been prepared by the Fries rearrangement of the propionate ester 3.24.[155] It was methylated to the ether but not reduced to the 2-methoxy-5-n-propylbenzoic acid 3.22. Cox also carried out a Fries rearrangement of the propionate of methyl salicylate.[156] Although no assignment was made for the product, the properties appear to correspond to the ortho-migration product, 3.26.

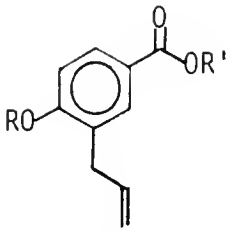


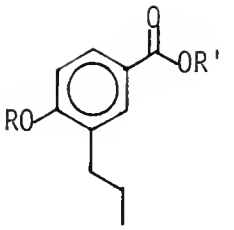
3.24 3.25, R = H 3.26

Analogs of the acid 3.23 have been prepared by two routes. In one of these, ethyl 3-allyl-4-hydroxybenzoate 3.28 was obtained by Claisen rearrangement of the corresponding allyl ether 3.27.[157,158,159] Methylation gave the ether 3.30, which on hydrolysis gave the acid 3.31.[157] Hydrolysis and reduction of 3.28 gave the propylanisic acid 3.29.[159] However, neither the methoxy derivative 3.23 nor its ester 3.31 was prepared.

The second route requires acylation of p-hydroxybenzoic acid to give the ketone 3.34, followed by reduction of the side chain. Fries rearrangement of the propionyl ester 3.32 gave 3.34,[160] but this compound was not reduced.







3.27, R = $CH_2CH = CH_2$, R' = C_2H_5

3.28, R = H, R' = C_2H_5

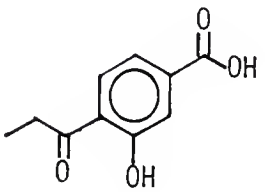
3.29, R = R' = H

3.30, R = CH_3, R' = C_2H_5

3.31, R = R' = CH_3

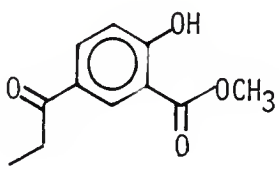
3.32, R = $COCH_2CH_3$, R' = H

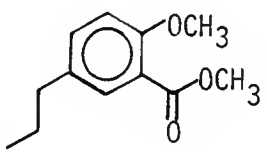
3.33, R = CH_3, R' = H



3.34

In the present work, Friedel-Crafts reaction of methyl salicylate with propionyl chloride gave methyl 5-propionyl salicylate (3.35).[a] This was reduced by the Clemmensen method to methyl 5-n-propylsalicylate and methylated to methyl 2-methoxy-5-n-propyl benzoate (3.36)

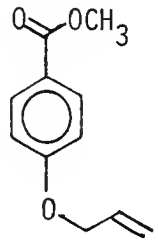




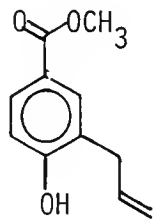
3.35

[a]The melting point of 5-n-propylsalicylic acid 3.25 obtained by hydrolysis of this sample is much greater than obtained by Cox[156] (210.5-213 vs. 177-9°C), supporting the contention that Cox has obtained the wrong isomer.

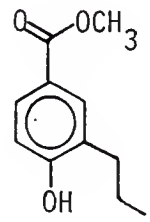
For the synthesis of the ester of 3.23, the allyl ether of methyl p-hydroxybenzoate 3.37 was subjected to Claisen rearrangement to methyl 3-allyl-4-hydroxybenzoate 3.38. This was reduced to the n-propyl derivative 3.36 and methylated to methyl 4-methoxy-3-n-propyl benzoate 3.31.



3.37



3.38



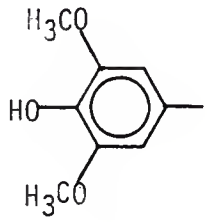
3.39

With the availability of the authentic samples of the two esters 3.36 and 3.31 which correspond to the acids 3.22 and 3.23, a comparison was made of the product of ozonolysis, brief permanganate treatment and esterification with diazomethane of the tetrahydro honokiol monomethyl ethers. Ether-A (higher R_f), which was identical with the tetrahydro derivative of the natural product, gave as product 3.31 while ether-B (lower R_f) gave 3.36. On the basis of these results, the structure of the natural product can be represented as 3.19.

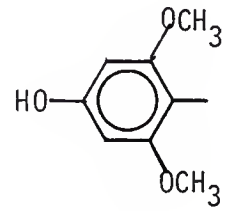
Lignan from the Wood

From the lipophilic phase of the wood extract of Magnolia grandiflora was isolated a crystalline solid, melting point 180-82°C $[\alpha]_D$ = +4.0° (c 1.12,$CHCl_3$). Its characteristic uv spectrum, λ_{max}

270 nm, log ε 3.41; 240 nm, log ε 4.21, and a base-induced shift of the maximum indicated that it was a phenolic substance. The mass spectrum (M+ 418) and elemental analysis agreed with the molecular formula $C_{22}H_{26}O_8$. The nmr spectrum showed the presence of four equivalent methoxyl groups and four equivalent aromatic protons. The presence of two phenolic hydroxyls was deduced from the formation of a diacetate (ir: 1763 cm^{-1}; nmr 2.32 ppm, 6H, s) and a dimethyl ether, which showed nmr signals for six methoxyl groups. The aromatic portion of the nmr spectrum with two equivalent protons on each ring may be ascribed to one of the two alternative structures:



3.39



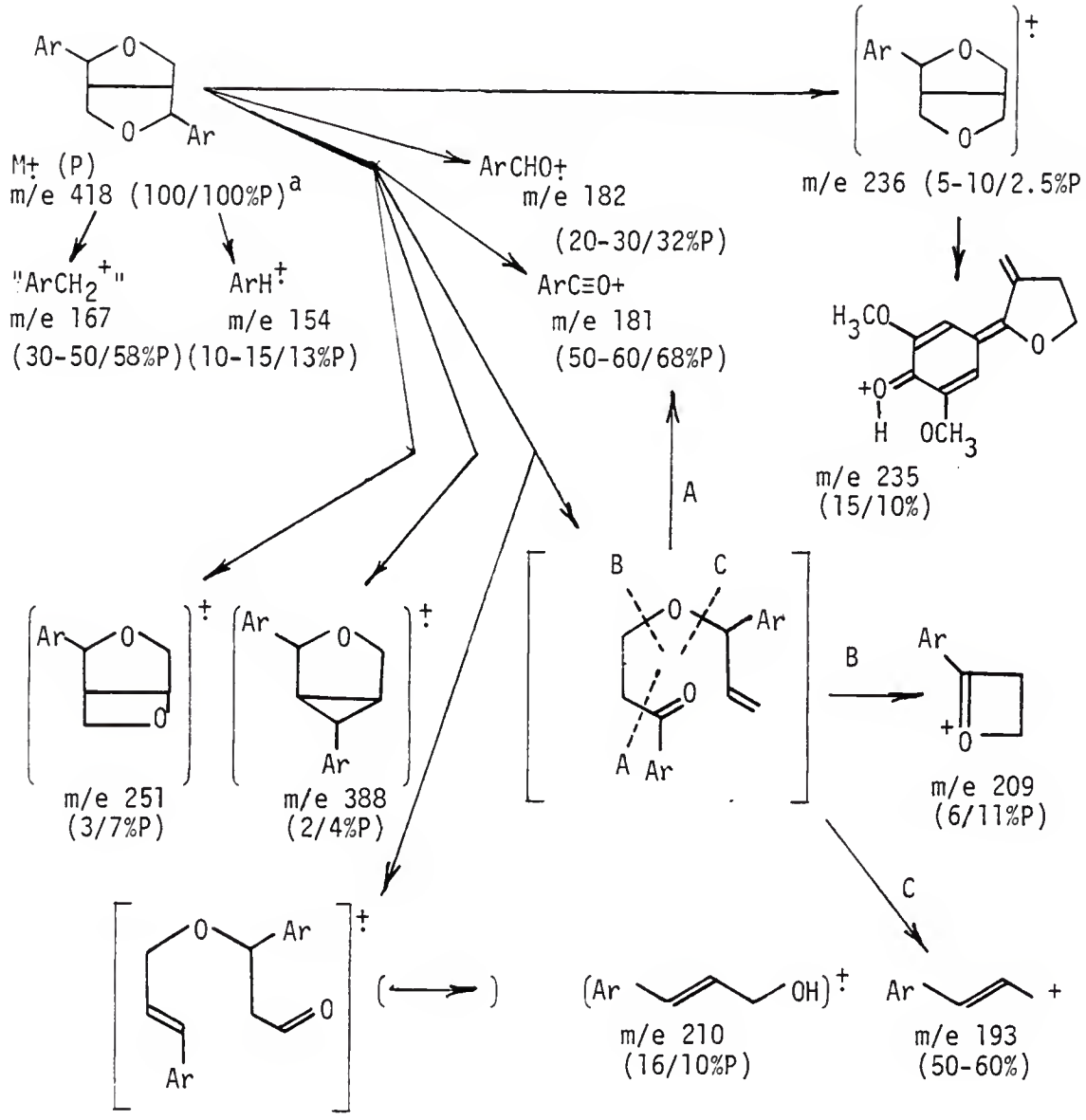
3.40

On the basis of the molecular formula and other nmr spectral characteristics which suggest a relationship to the lignoids, the syringyl residue 3.39 is much more consistent with the biogenetic origin of this class of compounds. Two syringyl residues are therefore considered to be present in the structure. The nmr spectrum, an analysis of which will be presented later, also suggested that the compound might possess a furofuranoid skeleton as found in lignans of the pinoresinol type.

Confirmation for the furofuran type structure was obtained through the analysis of the mass spectrum along the lines described by Pelter for pinoresinol.[161] A list of the major peaks and their probable origins and structures is shown in Figure 3.1. The close correspondence of the observed peaks with those expected for such a system showed that the Magnolia lignan is one of the diastereomeric lirioresinols and it remains to determine which of the three stereochemical representatations shown in Figure 3.2 is the correct one for the compound. (Relative stereochemistries were determined by Briggs, Cambie, and Couch.)[162]

Although three more isomers with a trans fusion of the furofuran ring system are theoretically possible, no examples of such a lignan have been isolated to date. The representation of the three known lirioresinols is due to Dickey et al., who first isolated two of these lirioresinols (A and B) by the acid hydrolysis of liriodendrin, a glycoside from Liriodendron tulipifera L. (Magnoliaceae) as well as from commercial beechwood sulfite liquors (Populus spp., Salicaceae).[164] Of these, syringaresinol corresponds to dl-lirioresinol B.[162] Emulsin-hydrolysis of liriodendrin gave an aglycone which was first assigned the structure of lirioresenil C[163] but was later shown to be a mixture containing lirioresinol B as the major component.[162] A compound of the structure of lirioresinol C has not yet been isolated from a natural source, although its dimethyl ether has been obtained from Macropiper excelsum (Forst. f.) Miq. (Piperaceae).[162]

The confusion that exists with lirioresinols is partially due to the lack of reliable chromatographic and spectroscopic information together with the variation in melting points probably arising from



Phenol and Phenol Ether Processes

M^{+}-15(CH_3) — m/e 403 (2%P)

M^{+}-28(C≡O) — m/e 390 (0%P)

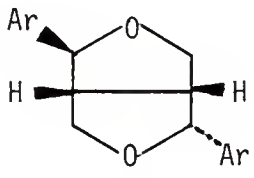
M^{+}-29(CHO·) — m/e 389 (1%P)

M^{+}-30(CH_2O) — m/e 388 (4%P)

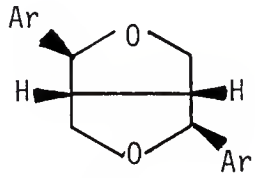
[a]Intensity of peak from Magnolia lignan/intensity of corresponding peak of pinoresinol

Figure 3.1 Comparison of mass-spectral fragmentation patterns of Magnolia lignan and pinoresinol

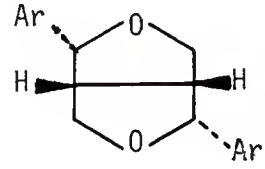
different enantiomeric compositions.[162,164] The physical properties of the three lirioresinols and their derivatives as reported in the literature are summarized in Table 3.1. Although the data from the table indicate that the lignan in question is indeed identical with syringaresinol B, there is sufficient variation in the literature data that such a conclusion may not be unequivocal, and additional evidence is desirable.



3.41
Lirioresinol A
(axial/equatorial)



3.42
Lirioresinol B
(diequatorial)



3.43
(Lirioresinol C)
(diaxial)

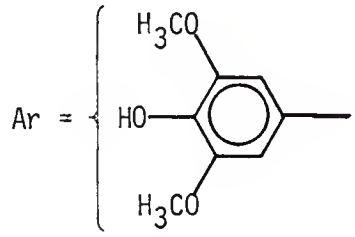


Figure 3.2 Relative stereochemistries of the lirioresinols

The infrared spectra (KBr pellets) of the Magnolia lignan and of synthetic syringaresinol[163] (cf. Figure 3.3) are practically identical and differ sufficiently from that of lirioresinol A that the latter may be excluded from consideration. However, such solid state spectra may not be completely defendable, as the formation of a racemic compound

Table 3.1 Properties of the lirioresinols and their derivatives

Compound	mp, °C	$[\alpha]_D^\circ$	Reference
Lirioresinol A	210-211	+127	163
	180-181	---	164
	177	-90	165
Lirioresinol A dimethyl ether	118-120	+119	163
Lirioresinol A diacetate	188	---	164
Lirioresinol B	177-183	-34.8	166
	175-179[a]	---	167
	172-179	+62.2	163
	170	-21.5	165
Lirioresinol B dimethyl ether	126.5-127	---	59
	122-123	+46.2	163
	122-123	+45.8	168
	121-122	+46.0	166
	116-118[a]	---	167
	115-119[a]	---	169
Lirioresinol B diacetate	188	---	163
Syringaresinol (dl-lirioresinol B)	179-185.5	0	170
	175	+1.93	171
	174	0	172
	170-171	0	173
	168-172	+3.93	164
Syringaresinol dimethyl ether	107-9	-4.14	171
	107-8[b]	0	172
Syringaresinol diacetate	188-189	-4.73	171
	181-182	---	164
	181-182	0	173
Lirioresinol C[c]	---	---	---
Lirioresinol C dimethyl ether	145-147	+284	162
Magnolia lignan	180-182	+4.0	this work
Magnolia lignan dimethyl ether	107.5-108	---	this work
Magnolia lignan diacetate	185-186	0	this work

[a] Assignment unclear

[b] Synthetic

[c] Genuine--many reports of lirioresinal C are incorrect

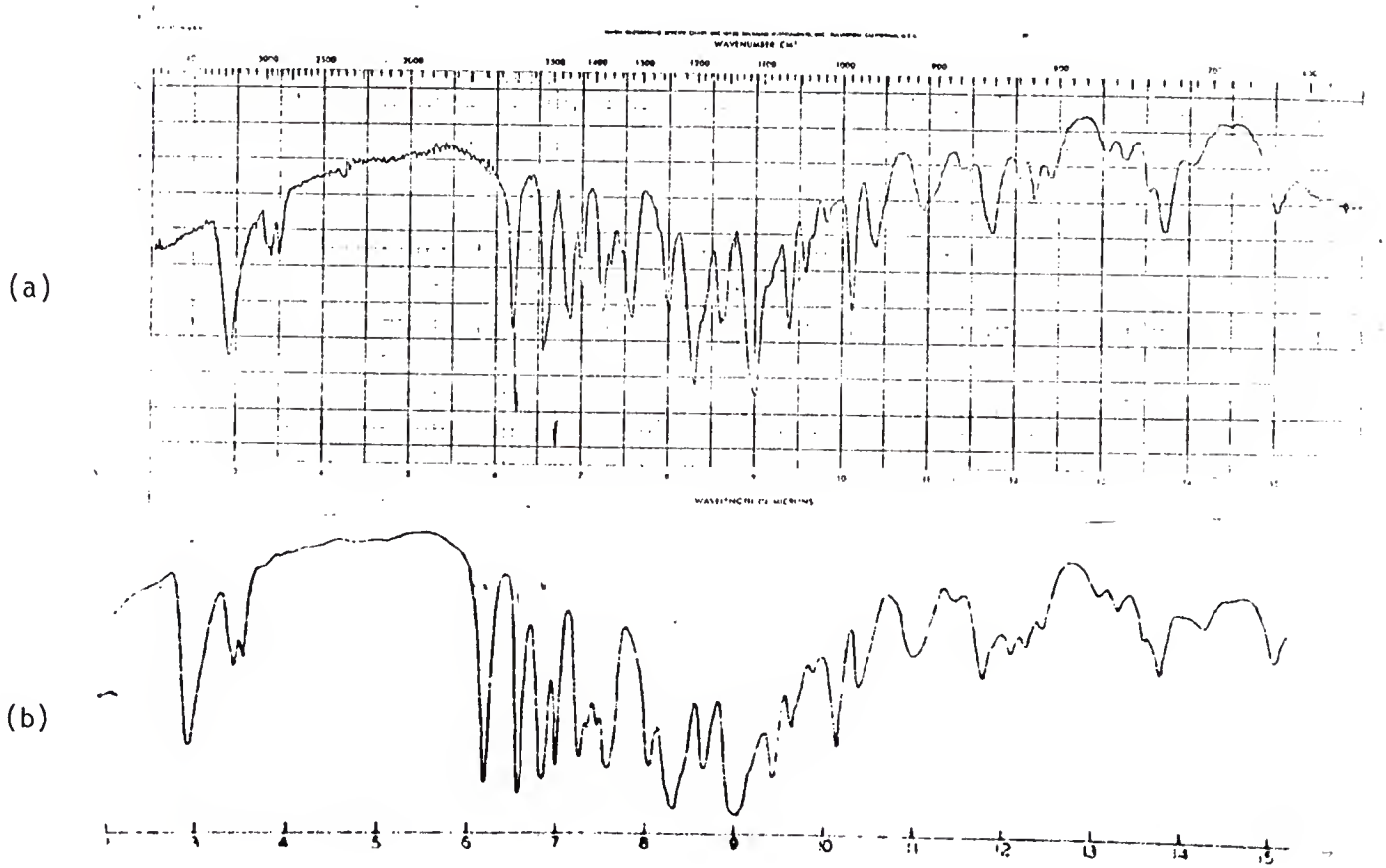
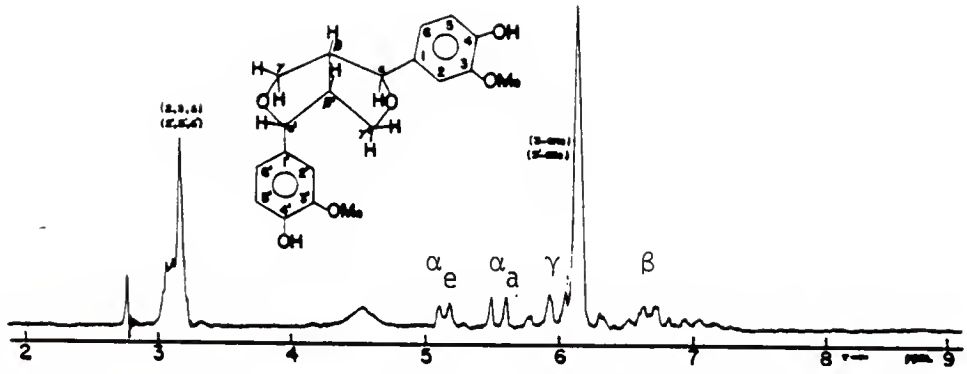


Figure 3.3 Infrared spectra (KBr) of (a) Magnolia lignan and (b) synthetic syringaresinol

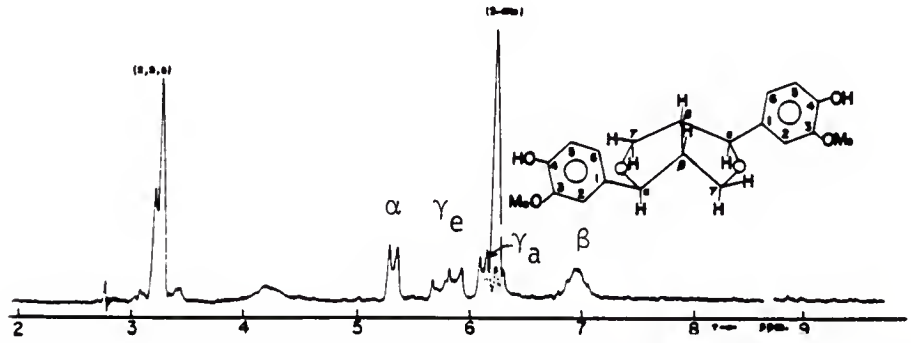
may alter the infrared spectra according to the enantiomer content.[174]

The nmr spectra of lirioresinols A and B are not available, although the corresponding isomers in the pinoresinol series are.[175] The two spectra are presented in Figure 3.4 and the spectrum of the Magnolia lignan is shown in Figure 3.5. General similarities between the spectrum of Magnolia lignan and that of (+) pinoresinol (the stereochemistry of which is firmly established),[176] are clearly apparent as are differences from that of (+)-epipinoresinol (same stereochemistry as lirioresinol A). Additional evidence that the Magnolia lignan possesses equatorially disposed aryl groups is obtained from assignment of these resonances.

In lirioresinol B, where both α-protons are axially disposed, the resonance appears at 4.75 ppm. In lirioresinol C, where both protons are oriented equatorially, they absorb at 4.98 ppm, while in lirioresinol A, the axial proton absorbs at 4.41 ppm, and the equatorial proton resonance appears at 4.83 ppm. This reflects greater shielding of the axial protons in the presence of an endo-aryl group.[162] In the case of the Magnolia lignan, the α-protons show a single resonance at 4.75 ppm, indicating that both protons are axially disposed; thus the Magnolia lignan has the same relative stereochemistry as lirioresinol B, which has been definitively shown to have a diequatorial disposition of the aryl groups by x-ray studies.[177] Assignments of these resonances based upon $J_{\alpha,\beta}$ are in error: it has been stated that in lignan spectra, overdependence on coupling constants, based upon bond

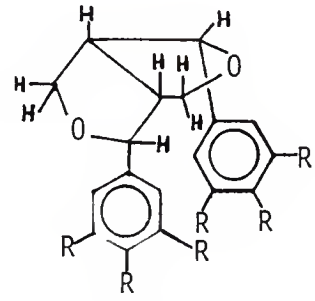


(a) Nmr spectrum of (+)-epipinoresinol (stereochemistry of lirioresinol A)



(b) Nmr spectrum of (+)-pinoresinol (stereochemistry of lirioresinol B)

$\delta H\alpha$ 4.98 ppm (d, J = 5 Hz)
$\delta H\beta$ 3.25 ppm
$\delta H\gamma_{eq}$ 3.63 ppm
$\delta H\gamma_{ax}$ 3.72 ppm



(c) Spectral assignments for lirioresinol C dimethyl ether (R = OCH_3)

Figure 3.4 Nmr spectra of the three pinoresinol lirioresinol skeleta

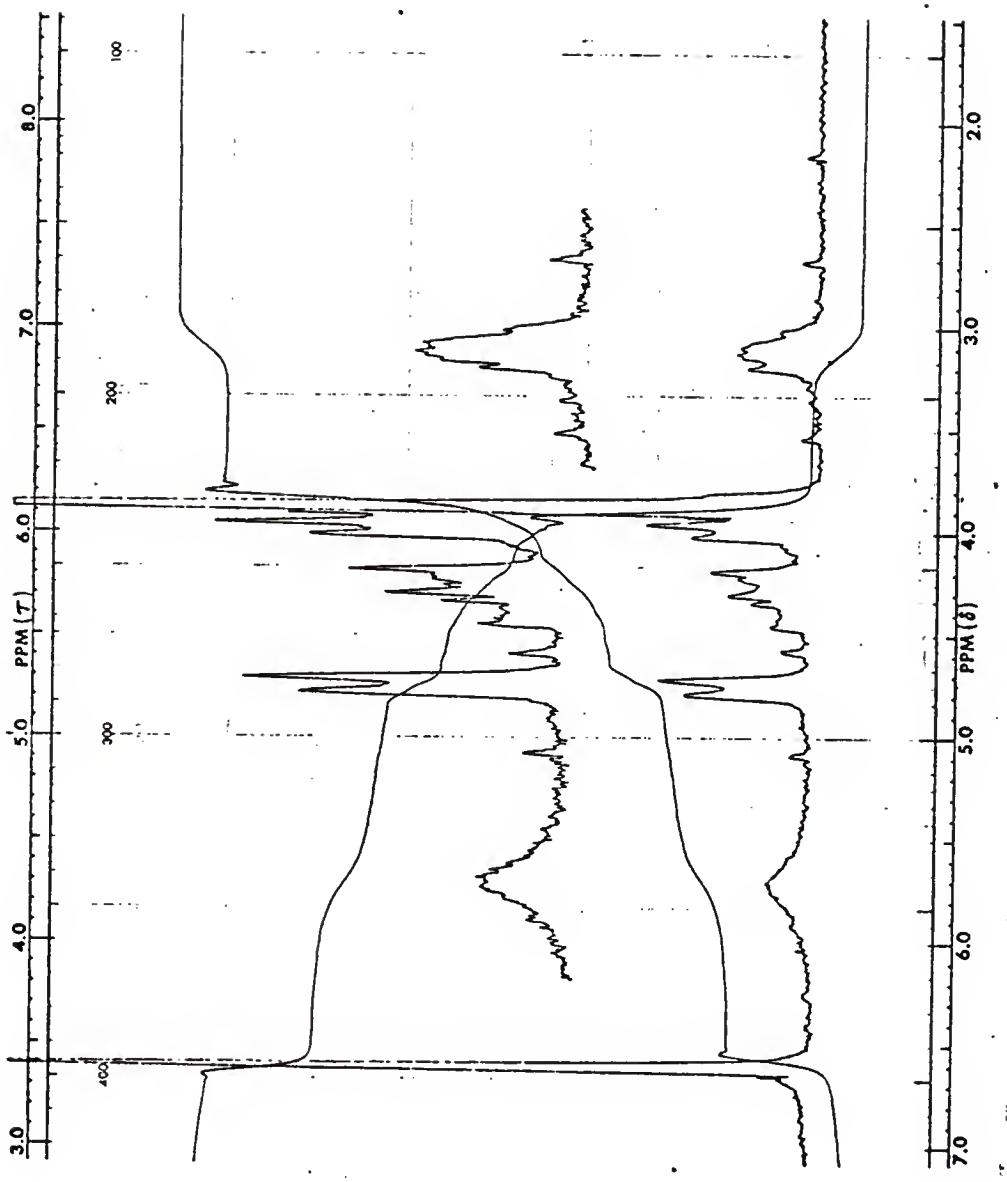


Figure 3.5 Nmr spectrum of Magnolia lignan ($CDCl_3$)

angles is undependable.[178,179] More emphasis is placed upon such shielding effects in making assignments.[162,178,180]

The isolation of syringaresinol from Magnolia grandiflora constitutes the first report of its presence in any species of Magnolia. The dimethyl ether has been isolated from Magnolia Kobus[59] and Magnolia (or Michelia) Fargesii. All lignoids isolated from Magnolia spp. to date are of three types: pinoresinol type, the galgravin type, (cf. Figure 1.3) and the bisallylphenol neolignans. As a chemical entity, syringaresinol was known long before its isolation from a natural source, having been obtained through oxidative, enzymic coupling of sinapyl alcohol.[172,181] Subsequently, it was synthesized by nonenzymic oxidative coupling of sinapyl alcohol and sinapic acid.[182] In the latter synthesis, the dilactone initially produced was reduced and pyrolyzed to yield syringaresinol.

Syringaresinol glycosides occur in a number of plants, including Magnolia grandiflora,[49] and Liriodendron tulipifera L.[163] (Magnoliaceae). Syringaresinol itself, as well as the optically active form lirioresinol B, has been obtained from Liriodendron tulipifera[167,169] (Magnoliaceae) Populus spp.,[164] Fagus spp.[173,182] (Betulaceae), Picea excelsa Engelm.[183] (Pinaceae), Sinomenium acutum Rehd. et Wils.[171] (Menispermaceae), and Xanthoxylum inerme Koidz.[170] (Rutaceae). The dimethyl ether has been obtained from Liriodendron tulipifera L.[167,169] (Magnoliaceae), Eremophila glabra[168] (Myoporaceae), Macropiper excelsum (Forst. f.) Miq.[162] (Piperaceae), and Aspidosperma Marcgravianum Woodson[166] (Apocynaceae), in addition to the Magnolias previously cited.

Experimental

Isolation of Mehonokiol 3.19

Ground bark (4 kg) of Magnolia grandiflora, collected in Gainesville, Florida, was extracted with ethanol (3 ℓ) at 25°C for two days. Three such extracts were combined and concentrated to a syrup which was partitioned between water and ethyl acetate (1 ℓ each). Concentration of the solvent extract gave a heavy oil (120 g).

Ten-gram portions of the oil were chromatographed on silica gel (250 g) in benzene. The benzene eluates contained, besides carotenoid pigments, a phenolic component (tlc: R_f 0.66 in silica benzene; positive diazo coupling). Fractions containing this were combined, concentrated and partitioned between hexane and NaOH (0.1N) in methanol:water (1:1). The aqueous alcoholic layer was concentrated, acidified (pH 2) and extracted with ether. The product from the ether was rechromatographed on silica gel using benzene:hexane (1:1). Fractions of the major band on concentration gave a colorless, heavy oil which was homogeneous on tlc and gc; yield, 0.2 g, 0.005%; uv: λ_{max} 255 nm (log ε 4.1) and 290 nm (log ε 3.8); with base, λ_{max} 320 nm; ir (neat, cm^{-1}) 3560, 3400-3200, 3090, 3010, 3000-2900, 2860, 1630, 1600, 1490, 1480, 1455, 1430, 1425, 1275, 1240, 1170, 1130, 1115, 1045, 1025, 987, 905, 810, 780, 725: nmr (ppm): 7.22-6.65, m, 6 ArH; 6.32-5.55, m, 2 $CH_2\underline{CH}=CH_2$; 5.81, m, 4.92, m, 2 $CH_2CH=\underline{CH_2}$; 4.86, s, exchangeable ArOH; 3.85, s, OCH_3; 3.40 and 3.28, overlapping doublets, J = 6 Hz, 2 $Ar\underline{CH_2}CH=CH_2$; ms: 280.1456; calc. for $C_{10}H_{20}O_2$: 280.1464.

Mehonokiol Acetate

A mixture of 3.19 (0.05 g), acetic anhydride (1 ml) and pyridine (two drops) was heated at 70°C for ten minutes. It was cooled and diluted with water. After 15 minutes, it was extracted with ether, the extract washed successively with dilute acid and aqueous sodium bicarbonate, concentrated to dryness and purified by a short silica gel column (5 g) in 2:1 hexane/benzene. The major fraction was obtained as a colorless, heavy oil: ir: 1755 cm^{-1}; nmr: 2.01 ppm, s, 3H; ms: M+ 322.1566, calc. for $C_{21}H_{22}O_3$: 322.1570.

Dimethylhonokiol

A mixture of 3.19 (0.1 g), methyl sulfate (0.1 ml) and anhydrous potassium carbonate (0.5 g) in acetone (5 ml) was stirred for 15 hours and filtered. The filtrate and wash were concentrated to dryness and purified by chromatography on silica gel (5 g) in 1:1 benzene/hexane to give the methyl ether as a colorless oil, ir: no OH; nmr (ppm); 3.71, s, 3H; 3.68, s, 3H; ms: M+ 294.1616; calc. for $C_{20}H_{22}O_2$: 294.1621.

Tetrahydromehonokiol 3.21

A solution of 5 (0.1 g) in ethanol (5 ml) was hydrogenated in the presence of PtO_2 in a Parr apparatus at 30 psi for one hour. The mixture was filtered and the filtrate concentrated to dryness to yield 3.21 as a colorless oil; ir: no bands at 1620, 985 or 905 cm^{-1}; nmr (ppm); 240 and 2.62 ppm, overlapping triplets, J = 5.2 Hz, 2 $ArCH_2CH_2CH_3$; 1.63 and 1.67 ppm, overlapping sextets, J = 8 Hz, 2 $ArCH_2CH_2CH_3$, 0.94 and 0.97 ppm, overlapping triplets, J = 8 Hz, 2 $ArCH_2CH_2CH_3$; ms: M+ 284, m/e 255, 223, 205.

Anal. Calc. for $C_{19}H_{24}O_2$: C, 80.25; H, 8.52. Found: C, 80.24, H, 8.80.

4-Iodo-2-propylanisole 3.17

o-Allylphenol (Aldrich Chemical Co., 6 g) was hydrogenated as described for 3.21 and methylated in acetone (200 ml) with methyl sulfate (10.5 ml) and potassium carbonate (15 g), stirring at room temperature for 36 hours. The product, 2-propyl anisole (10 g) was iodinated by the method of Hata and Satō[153] using mercuric oxide-catalyzed iodination by I_2 in ethanol. The resulting product was purified by distillation (1.5 mm, 105-115°C) to give 3.13; yield 10 g (90%).

2-Iodo-4-propylanisole 3.16

Anethole (Aldrich Chemical Co., 10 g) was hydrogenated and the product iodinated as above. The iodinated product was purified by distillation (1.5 mm, 100°C); yield 8 g (88%).

Tetrahydromagnolol dimethyl ether 3.5

The coupling of 3.16 (10 g) was carried out by a modification of the procedure of Fujita et al.,[55] in which the copper powder (22 g) was gradually added to 3.16 at 240°C. After completion of the addition, the mixture was maintained at this temperature for four to six hours instead of heating to 285°C. This change gave a better quality product and a higher yield. Purification was by chromatography on silica gel. Using benzene:hexane (1:1) as the eluent 3.5 was obtained as a colorless oil, 3 g; yield, 56%; uv: 287 nm (log ε 3.7),

256 nm (log ε 4.0); nmr (ppm): 6.70-7.20, m, 6 ArH; 3.68, s, 2 OCH_3; 2.50, 1.60 and 0.95, triplet/sextet/triplet, respectively, 2 $CH_2CH_2CH_3$.

Demethylation of 3.5 (1 g) with acetic anhydride (10 ml) and hydriodic acid (8 ml) gave tetrahydromagnolol, yield 0.5 g (64%); mp 142-142.5°C (lit. 143°C).[55]

4,4'-Bis(2-propyl)anisole 3.7

Coupling of 3.17 (8 g), carried out as described under 3.5, gave 3.7 as a colorless, crystalline solid; yield, 4.3 g (95%); mp 113-14°C (lit. 114°C).[55]

Demethylation of 3.7 (1.2 g) with hydroidic acid (7 ml) gave 4,4'-bis(2-propyl)phenol, yield, 1 g (97%); mp 113-114°C (lit. 112.5°C).[55]

Methylation of 4,4'-bis(2-propyl)phenol (0.75 g) in acetone (20 ml) with methyl sulfate (0.36 g) and potassium carbonate (1 g) at 25°C for 24 hours gave a mixture of products from which the monomethyl ether 3.24 was separated by chromatography (silica gel, hexane with benzene gradient) and crystallized from hexane; yield, 0.4 g (50%); mp 79-80°C; nmr: (ppm) 3.4-2.6, m, 6-ArH; 4.88, s, Ar-OH; 6.23, s, OCH_3; ms: M+ 284.

Dimethyltetrahydrohonokiol 3.6

Crossed Ullmann coupling of 3.16 and 3.14 was carried out using 6 g of each as described under 3.5. The product (gc: 3% OV-17, T 180°C, 3.5, t_r 11 min., 25%; 3.6, t_r 15.8 min., 55%; and 3.7, t_r 20.8 min., 19%) was subjected to chromatography on Florisil for preliminary purification. Direct crystallization gave 3.7 (0.9 g), the mother liquors were purified by chromatography (silica gel, cyclohexane with

a benzene gradient, 25-100%). The first two product bands contained 3.5 and 3.7 and the third band the desired 3.6, identical with the natural O-methyl tetrahydromehonokiol (gc as above; tlc: R_f 0.70, silica, benzene:hexane, 1:1); yield, 1.3 g. A total yield of 67% of the three coupled products (99% conversion) was obtained.

Demethylation of 3.6 (1 g) by hydriodic acid (10 ml) gave tetrahydrohonokiol 3.9; yield, 0.74 g (83%); mp 117-117.5°C (lit. 118°C).[53]

Mono-O-methyltetrahydrohonokiols 3.21 and 3.22

A solution of 3.9 (1.1 g) in acetone (25 ml) was stirred with methyl sulfate (0.64 g) and potassium carbonate (2 g) at 25°C for 48 hours. In addition to the dimethyl ether (40%), and the unreacted diol (20%), the two monoethers were formed in a yield of 20% each (gc: 3% SE-30, 200°C; 3.21 t_r 5.5 min.; 3.22 t_r 8.3 min., tlc: R_f 0.90, 0.60, respectively, silica benzene). The monoether of higher R_f was identical with the natural tetrahydromehonokiol by tlc, gc and spectral comparison. The mixture was separated by chromatography (silica gel, cyclohexane with a benzene gradient). The high R_f ether 3.21 was obtained as a colorless oil: ms: 284.1790 (calc. for $C_{19}H_{24}O_2$: 284.1775); m/e 269 (100%), 256 (17%) and 255 (80%).

Anal. Calc. for $C_{19}H_{24}O_2$: C, 80.24, H, 8.50. Found: C, 80.32, H, 8.71.

The low R_f ether 3.22 was obtained as a colorless oil; (spectral data similar); Anal. Calc. for $C_{19}H_{24}O_2 \cdot \frac{1}{2} H_2O$: C, 77.85; H, 8.59. Found: C, 77.69: H, 8.65.

Partial Demethylation of Tetrahydrohonokiol Dimethyl Ether

A mixture of 3.6 (50 mg) and aluminum chloride (50 mg) in nitrobenzene (50 mg) in nitrobenzene (5 ml) was stirred overnight, diluted to 20 ml (benzene) and washed three times with HCl (IN), extracted twice with brine. The phenols were extracted into 30% methanol/KOH (0.5), the extract neutralized and examined by gc as above: 3.20 and 3.21 were formed in equal amounts.

Ozonolytic Degradations

Ozone from a generator (Ozone Research and Equipment Co.) was bubbled into a solution of 3.24 (0.1 g) in N ethanolic potassium hydroxide (0.02N) at -30°C until the abosrbance value at 290 nm reached a stable value at approximately 10% of the original. The reaction mixture was maintained basic during this time with additional base when needed. The mixture was concentrated to remove the ethanol, the residue dissolved in water (10 ml) and treated with 5% aqueous potassium permanganate dropwise until the reaction became slugglish. It was then acidified (pH 1-2) and treated with sodium bisulfite until a clear, colorless solution was obtained, which was extracted twice with ether. The acidic product was recovered from this by washing with aqueous bicarbonate, followed by acidification of the aqueous layer and reextraction with ether. The product from the extract was esterified with diazomethane to the crystalline methyl ester, 35 mg, mp 35-37°C; underpressed when mixed with an authentic sample of methyl 3-n-propyl-p-anisate 3.31. The spectral (ir, nmr) and chromatographic were identical (tlc: R_f 0.60, silica, benzene; gc: 3% SE-30, 130°C, t_r 7 min.).

Similar degradation of 3.21 gave, after esterification, a product identical with methyl-3-n-propyl p-anisate 3.31 by mp, spectral, and chromatographic comparison.

Degradation of 3.22 and esterification gave a product identical with methyl-3-n-propylsalicylate 3.36 by spectral and chromatographic comparison (tlc: R_f 0.25, silica, benzene; gc: 3% SE-30, 130°C, t_r, 7.3 min.)

Methyl-3-n-propylsalicylate 3.36

Methyl salicylate (2 g) in nitrobenzene (10 ml) was treated with anhydrous aluminum chloride (1.7 g) and then with propionyl chloride (1.3 g), added dropwise at 0°C. After 16 hours at 5°C it was diluted with benzene and washed successively with 1N hydrochloric acid, water and 0.1N sodium hydroxide. The basic phase was acidified, extracted with ether and the product purified by chromatography (silica gel, 1:1 benzene:hexane (1:1)). Methyl-5-propionyl-salicylate 3.35 was obtained as a colorless crystalline solid, mp 61-62°C; yield, 0.5 g.

The sample was boiled under reflux with zinc amalgam (5 g) and 6-N hydrochloric acid (50 ml) for five hours. The cooled mixture was extracted with ether and the extract concentrated. Chromatography of the residue gave methyl-3-n-propylsalicylate 3.36 as a colorless oil; yield, 0.35 g; ir: 1730, 1610, 1255 cm^{-1}; nmr (ppm); 7.60, 72.6, 6.86, pattern of 1,2,4-protons on benzene, 3H; 3.86, s, 6H; 2.56, 1.62, 0.91, triplet/sextet/triplet; $ArCH_2CH_3CH_3$.

Saponification (1N KOH, reflux 5 min.) gave, on workup, the acid 3.25, mp 210-211.5°C from water (lit. 177-9°C).[156] (cf. p. 59).

Methyl-3-n-propyl-p-anisate 3.31

A mixture of methyl p-hydroxybenzoate (6 g) allyl bromide (5.2 g) and anhydrous potassium carbonate (8 g) in acetone was boiled under reflux for six hours. The cooled, filtered reaction mixture was concentrated to yield 4-carbomethoxy phenylallyl ether 3.37 as a colorless oil; yield, 6.8 g (90%); ir: 1720, 1610, 1000, 930 cm^{-1}; nmr (ppm) 7.96, 6.89, AB-pattern, 4H; 6.40-5.10, 4.60, m, $CH_2CH=CH_2$, 3.85, s, OCH_3.

A sample of 3.31 (5 g) was rearranged by heating (N_2) in phenyl ether (20 ml) at 220°C for five hours. After cooling, the phenolic product was separated by extraction with sodium hydroxide (0.1N) and recovered by acidification of the basic extract followed by extraction with ether. Concentration of the extract gave methyl 3-allyl-4-hydroxybenzoate 3.38 as a colorless oil, yield 4.5 g (90%); ir: 3600-3200, 1685, 1640, 1610, 990, 910 cm^{-1}; uv: 262 nm, log ε 4.2, shifted to 306 nm with base.

The sample was methylated in acetone by stirring with methyl sulfate (5 g) and potassium carbonate (8 g) for 15 hours at 25°C. The mixture was filtered and the product recovered by concentration of the filtrate and crystallization; mp 122.5-125°C. When subjected to catalytic hydrogenation in ethanol in the presence of Pt at 40 psi in a Parr apparatus, it gave methyl-3-n-propyl-p-anisate 3.31 as a colorless crystalline solid; mp 36-37°C. It was identical with the methylated ozonolytic degradation product of tetrahydromehonokiol, 3.21.

Saponification (1N KOH, refluxed 5 min.) gave on workup the acid, 3.29, mp 115-17°C from water (lit. 116.8°C).[159]

(dl)-Syringaresinol 3.43

The ethyl acetate phase of the wood extract (10 g) was taken up in ethyl acetate (50 ml) and extracted three times with KOH (0.01 N, 30 ml). The combined extracts were neutralized and reextracted with 130 ml chloroform. The combined chloroform phases were dried (Na_2SO_4), concentrated, the phenolic lignan extracted and isolated by chromatography (silica gel, 20 g, benzene with ethyl acetate solvent gradient). The lignan, (tlc: R_f 0.30, 5% methanol:chloroform), was eluted by 25% ethyl acetate, the lignan-containing fractions concentrated and crystallized from ether, affording 3.43; 60 mg, mp 180-182°C; $[\alpha]_D$ +4.0° (c 1.12 $CHCl_3$); uv: 220 nm, log ε 3.41; 240 nm, log ε 4.21 (lit. 270 nm/3.46, 237 nm/4.66),[164] ir, nmr: mp cf. Figures 3.3, 3.5, and Table 3.1.

Anal. Calc. for $C_{22}H_{25}O_8$: C, 63.14; H, 6.26. Found: C, 62.83; H, 6.34.

Syringaresinol Dimethyl Ether

The lignan 3.43 (100 mg) was methylated with dimethyl sulfate (0.5 ml) and anhydrous potassium carbonate (0.79) in acetone (15 ml), with vigorous stirring for 48 hours. The reaction mixture was filtered, the filtrate concentrated and the residual oil chromatographed on a 50 g silica-gel column (50 g). It was eluted with 2.5% acetone in benzene. The fractions of the band, on concentration, gave 100 mg (92% yield) of a colorless, crystalline solid, recrystallized from ether: mp 107.5-108°C; ms: cf. Figure 3.1; uv: 270 nm, log ε 3.40; 240 nm, log ε 4.21.

Anal. Calc. for $C_{24}H_{30}O_8$: C, 64.56; H, 6.77. Found: C, 64.41; H, 6.67.

Syringaresinol Diacetate

The lignan 3.43 (300 mg) was treated at room temperature with 3 ml of acetic anhydride and two drops of pyridine. After 48 hours, the solution was diluted to 20 ml with water. After one hour, the derivative was extracted into chloroform, the extract washed with 10% sodium bicarbonate, followed by brine, dried over sodium sulfate, and concentrated to dryness. The resulting oil was crystallized from ether, affording 3.43 diacetate, 320 mg, a colorless, crystalline solid, mp 185-186°C, $[\alpha]_D$ 0.0° (c 0.50, $CHCl_3$); nmr: 2.32 ppm, 6H, s, CH_3CO_2Ar; ir: 1764 cm^{-1}.

Anal. Calc. for $C_{26}H_{30}O_{10}$: C, 62.14; H, 6.02. Found: C, 61.87: H, 6.10.

CHAPTER 4
ALKALOIDS OF *Magnolia grandiflora* L.

Toxic Alkaloidal Fraction

The present study started with the observation that the extract of the wood of *Magnolia* was toxic to mice when administered by the intraperitoneal route. The major symptom of toxicity appeared to be respiratory paralysis. Since no prior reports existed regarding this activity, in spite of extensive studies on *Magnolia* spp., isolation of the active principle was justified.

Partition of the concentrate of the alcoholic extract between water at pH 2 or 9 and ethyl acetate showed the activity in the aqueous phase, thus showing that the active principle was water soluble. Since the aqueous layer gave a positive test for alkaloids, an aliquot of the concentrate was treated with Mayer's reagent, the alkaloidal principle separated from the nonalkaloidal components and both samples freed from the HgI_4^- ion and tested. The alkaloidal fraction was found to be toxic. Since extraction at pH 9 did not transfer the activity into the solvent layer, the alkaloid was considered to be quaternary.

For the isolation of the active alkaloid, precipitation with Mayer's reagent, followed by exchange of the HgI_4^- ion by Cl^- ion using a weak or strong base type ion-exchange resin was found to be the most convenient. At this point, extraction with a solvent such as chloroform was again attempted at pH 9 and found that one of the alkaloids was

extractable, although the activity still remained in the aqueous layer. The bulk of the extractable alkaloid was therefore removed by this method and purified as the crystalline hydrochloride. It was further observed that the toxic quaternary alkaloid(s) could be extracted partially into n-butanol. The n-butanol extract contained approximately 40% of the toxic fraction, the remainder being left behind in the aqueous layer. The recovery and purification methods employed are summarized in Figure 4.1.

Methods available in the literature for the purification of quaternary alkaloids are very few. They are generally processed via precipitation, regeneration to a salt such as Cl^- or I^-, and crystallized. When this is not possible, chromatographic methods have been used with silica gel or alumina. However, because of the necessity for the use of solvents of high polarity (e.g., 10-30% methanol in chloroform) the resolution is very poor. The situation is even more difficult when the quaternary alkaloid is also phenolic which results in an even stronger affinity for the adsorbent. The alkaloid in question from Magnolia appeared to be phenolic in nature as judged by the base-induced uv spectral shifts and very strongly adsorbed on silica in thin-layer chromatography unless very polar solvents were used.

A method based on chromatography on Sephadex LH-20 is employed in these laboratories for the purification of relatively water-soluble compounds such as glycosides, quaternary alkaloids and peptides. A combination of adsorption/partition processes is involved when a solvent such as ethyl acetate is used in combination with varying proportions

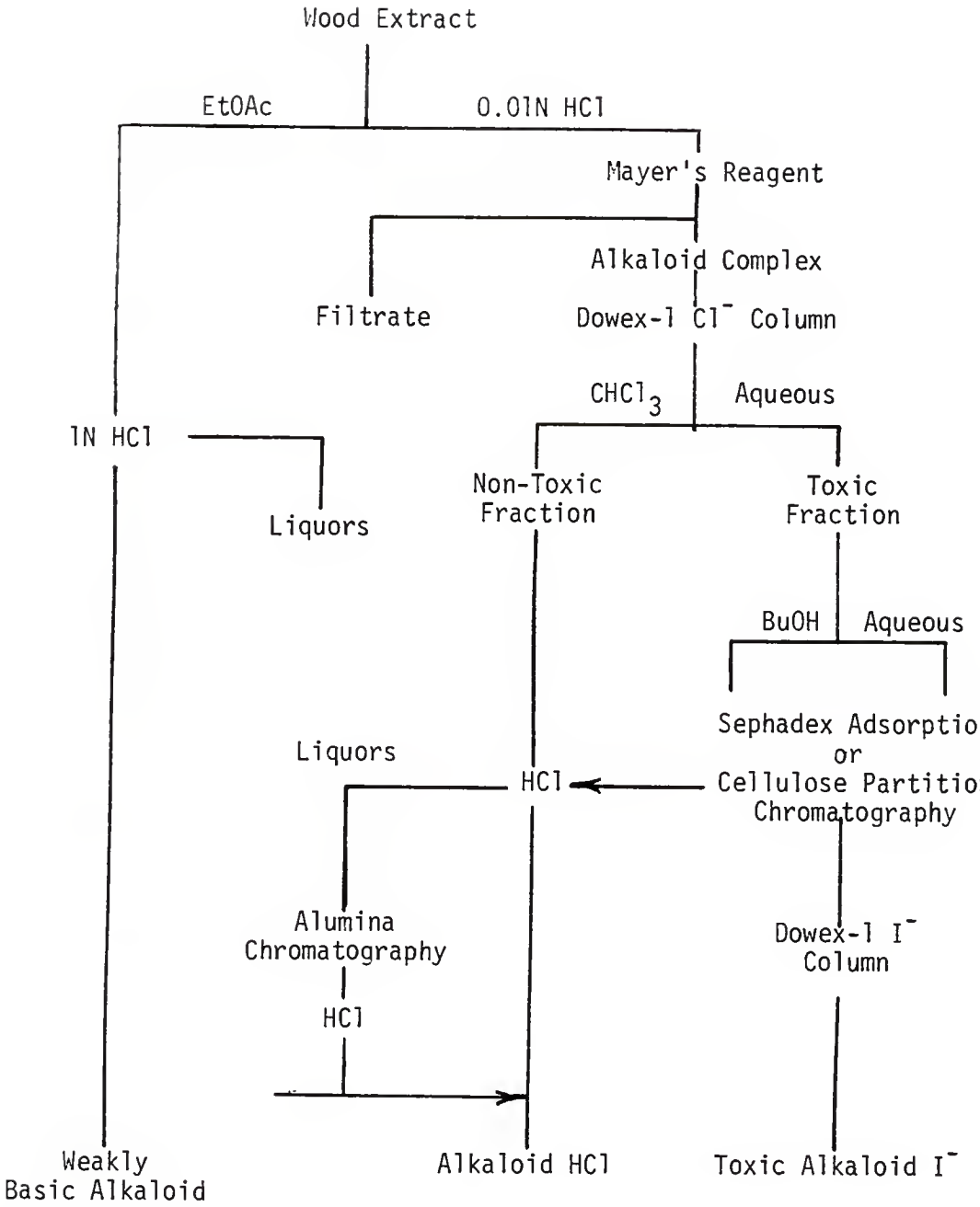


Figure 4.1 Fractionation scheme for Magnolia wood alkaloids

of ethanol and water. Unlike the conditions which exist in gel-permeation chromatography, the solutes are not eluted on the basis of their molecular size but are adsorbed on the basis of their polarity, aromaticity and such characteristics, and are eluted by the appropriate solvent. Also, unlike adsorption chromatography with silica gel or alumina, there is virtually no danger of loss of compounds due to irreversible adsorption.

By this procedure, the crude alkaloidal fraction from the n-butanol extract was purified using the solvent mixture 10% ethanol in ethyl acetate on Sephadex LH-20. Because of the low solubility of the sample in the solvent mixture, the initial chromatography gave broad elution peaks which appeared to contain different alkaloidal components. However, when these were rechromatographed on a second column, the elution profile was much sharper and more reproducible. Alternatively, partition chromatography on cellulose using the system ethyl acetate: n-butanol (3:1) and water was also satisfactory for the purification. Several alkaloidal fractions were recognized in the elution profile as shown in Figure 4.2. The first contained a compound which was the same as the one extractable by chloroform. The second and major peak represented the toxic, quaternary alkaloid. The final peak contained a small amount of a quaternary alkaloid which was characterized as magnoflorine, which is the major alkaloid of Magnolia grandiflora bark.[15]

The quaternary alkaloid was next converted to the iodide salt by ion-exchange using Dowex-1 iodide and was obtained as a crystalline solid, mp 222-25° (dec.); $[\alpha]_D$+212° (c 1.27, ethanol) representing a

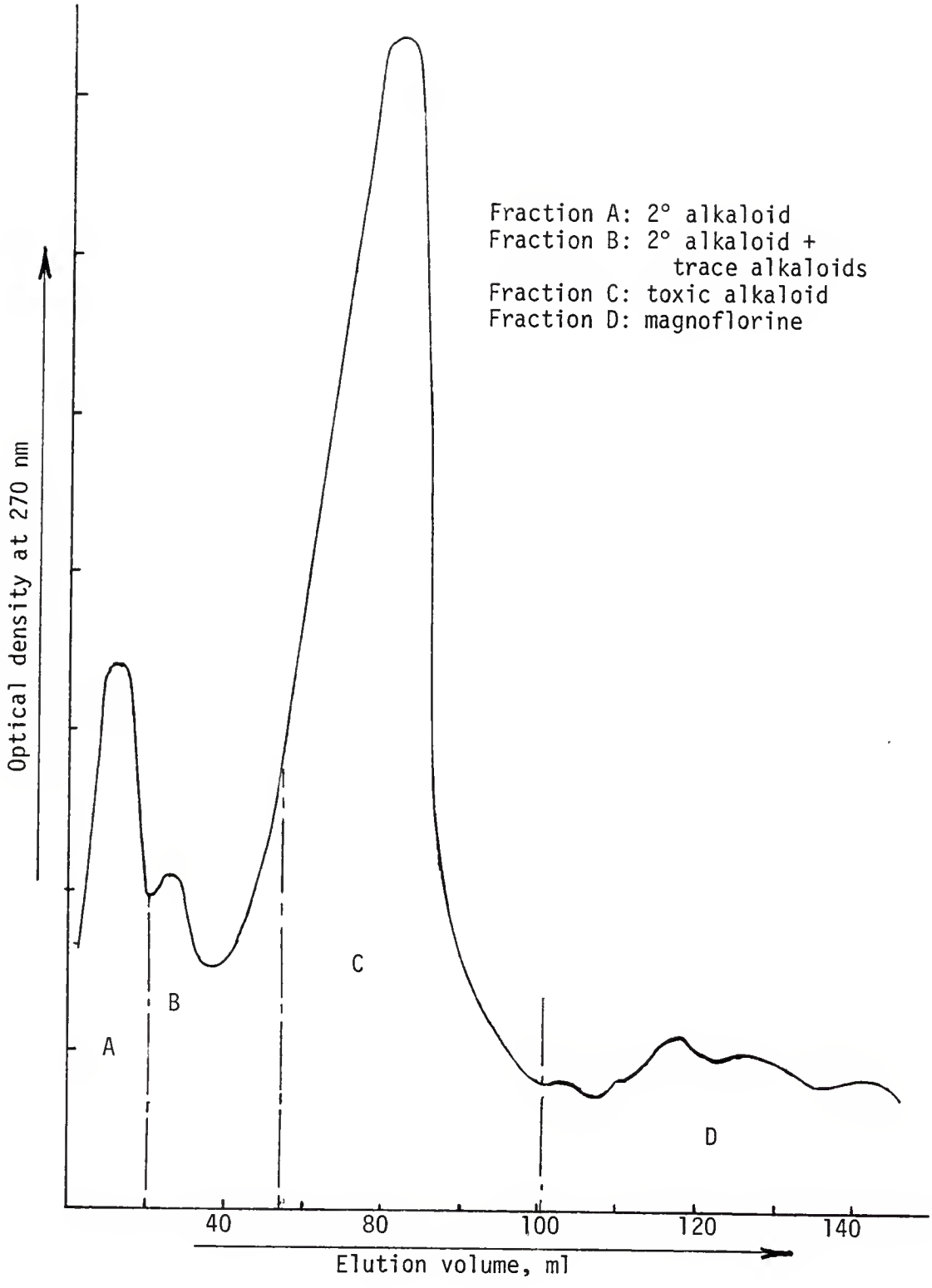


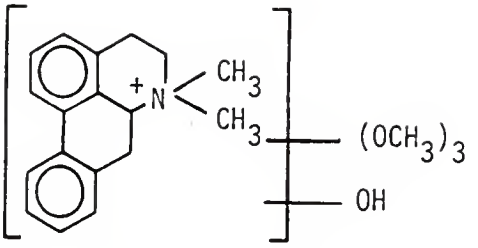
Figure 4.2 Cellulose partition chromatography of BuOH fraction

yield of 0.02%. It was also toxic to mice with an LD_{50} value of 10 mg/kg.

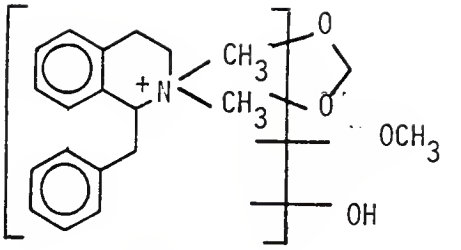
Elemental analysis of the toxic alkaloid agreed with the molecular formula $C_{20}H_{26}NO_4I$. Its uv spectrum showed λ_{max} at 220 nm, log 4.76; 270 nm, log 4.21 and 302 nm, log 3.82, with base-induced shifts to 250 and 354 nm. The ir spectrum showed bands for a phenolic hydroxyl, 3200 cm^{-1}, 1230 cm^{-1} and for an aromatic system, 1600 cm^{-1}.

Quaternary alkaloids generally undergo some form of dequaternization in the mass spectrometer by one of the following processes: nucleophilic attack by the counterion on the methyl group which leads to a loss of CH_3X, or a Hofmann elimination of HX. The actual course depends on the alkaloid and the nature of X:[184] with Magnolia alkaloids and $X = I^-$, the former predominates to give a spectrum identical to that of the norbase. The alkaloid in question gave a molecular ion m/e 341 corresponding to that of the norbase, and methyl iodide m/e 142, with lesser peaks at m/e 298 ($M^+ - CH_2{=}NCH_3$), m/e 326 ($M^+ - CH_3$), and m/e 310 ($M^+ - OCH_3$).

The data suggested that the Magnolia alkaloid might possess either an aporphine system or a benzyltetrahydroisoquinoline system with a methylene dioxy group as shown in 4.1 or 4.2. The nmr spectrum which showed signals at 8.67 ppm, broad, exchangeable, Ar-OH; 7.00 ppm, 1H; 6.98 and 7.11 ppm, AB-q, J = 8 Hz, 3.72, 3.85 and 3.93, singlets each equal to $3H(OCH_3)$; 3.44 and 2.98 singlet each equal to $CH_3(NCH_3)$ clearly eliminated a structure such as 4.2, and indicated the presence of 3 methoxyl and 1 hydroxyl groups. Other lines of evidence such as the mass spectrum, which did not show significant peaks to correspond

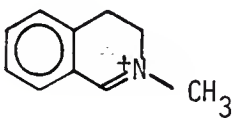


4.1



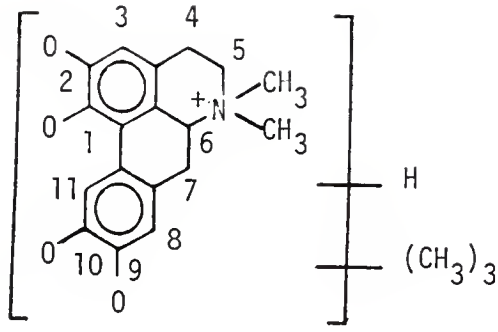
4.2

to an iminium ion such as 4.3 derived by the α-fission of a benzyltetrahydroisoquinoline system[185] and the uv spectrum[186,187] established that the alkaloid has an aporphine skeleton 4.1.

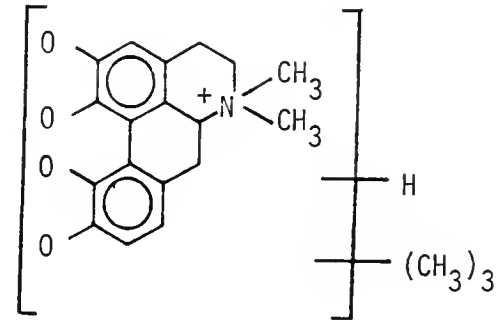


4.3

The presence of a phenolic hydroxyl was verified by the formation of a monomethyl ether, nmr: 3.71 ppm, 6H; 3.90 ppm, 3H and 3.92 ppm, 3H. Acetylation with acetic anhydride and pyridine did not proceed, possibly due to steric hindrance, but acid-catalyzed acetylation gave an acetate, ir: 1770 cm^{-1} and nmr: 2.10 ppm. Thus, the alkaloid is a hydroxytrimethoxyaporphine with the oxygenation pattern yet to be determined. On a biogenetic basis, only two oxidation patterns are likely: 1,2,9,10 (4.4) or 1,2,10,11 (4.5).



4.4



4.5

The former may be eliminated by spectroscopic evidence from several sources. The aromatic region of the nmr spectrum shows an AB-quartet consistent with the presence of two ortho-protons which can only be explained by 4.5. The uv spectrum of the Magnolia alkaloid: 220, 270 and 302 nm is typical of a 1,2,10,11-oxygenated aporphine and not of a 1,2,9,10 system, which has maxima at 280-283 nm and 302-310 nm.[186,187] This difference has been said to reflect the greater interference with coplanarity of the biphenyl system in a 1,2,10,11-oxygenation pattern.[187] The mass spectra of several 1,2,9,10-oxygenated aporphines have been published[185,186,188] and the characteristic processes are summarized in Figure 4.3. Chiefly, these include (a) α-cleavage to an iminium ion such as 4.7 which appears as the molecular ion, or through the loss of H· to 4.6 (M^+ -1), (b) subsequent formation of systems such as 4.8 or 4.9 with the loss of either $\cdot OCH_3$ or $\cdot CH_3$, respectively, and, (c) retro Diels-Alder cleavage to 4.10 which can undergo further loss of $\cdot OCH_3$ or $\cdot CH_3$.[185,188] In contrast, the mass spectum of the Magnolia alkaloid showed no M^+ -1 peak, low intensity

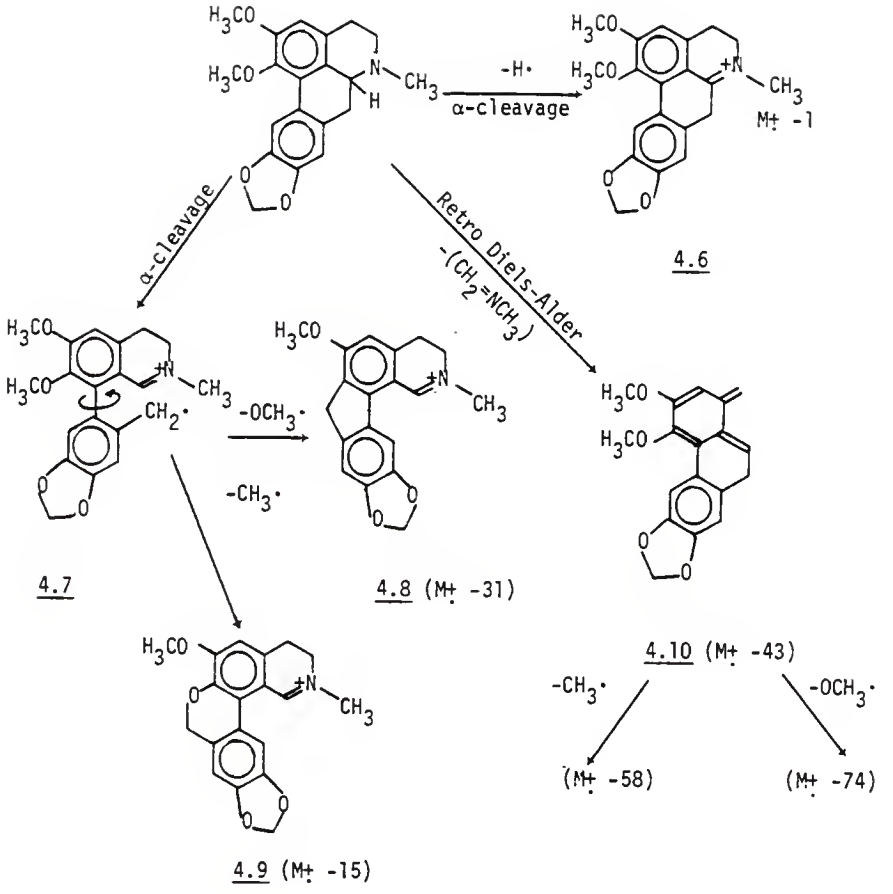
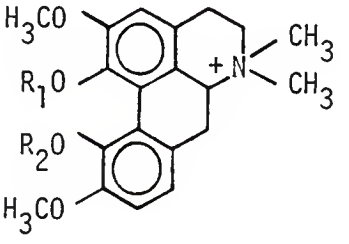


Figure 4.3 Mass spectral behavior of a typical aporphine

M^+ -43 peak (retro Diels-Alder process) but a relatively high intensity M^+, M^+ -OCH_3 peaks. Such behavior is peculiar to those aporphines with 1,2,10,11-oxygenation pattern.[189]

Final proof for the 1,2,10,11-oxygenation pattern was obtained through methylation of magnoflorine 4.10. The resulting dimethylmagnoflorine 4.11 was found to be identical with the methyl ether of the Magnolia alkaloid by comparison of its tlc and spectral behavior.

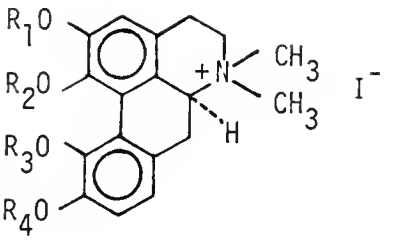


magnoflorine 4.10 $R_1 = R_2 = H$

dimethyl magnoflorine 4.11 $R_1 = R_2 = CH_3$

For the Magnolia alkaloid, four structural possibilities exist for the location of the phenolic hydroxyl, as shown in Figure 4.4. Of these, neither 4.12 nor 4.15 has been isolated from natural sources so far, while 4.13, N-methyl corydinium iodide and 4.14, N-methyl isocorydinium iodide are known.

The difficulty with acetylation of the phenolic hydroxyl might suggest that it be present at C-1 or C-11. This is further supported by the hydroxyl frequency of 3200 cm^{-1} in the ir spectrum. Aporphines of this substitution pattern, as well as the phenanthrenes derived from



N-methyl-10-O-methylhernovine methiodide	4.12 $R_1 = H_1$, $R_2 = R_3 = R_1 = CH_3$
N-methyl corydinium iodide	4.13 $R_2 = H_1$, $R_1 = R_3 = R_4 = CH_3$
N-methyl isocorydinium iodide	4.14 $R_3 = H_1$, $R_1 = R_2 = R_4 = CH_3$
----------	4.15 $R_4 = R_4 = H_1$, $R_1 = R_2 = R_4 = CH_3$

Figure 4.4 Isomeric hydroxy-trimethoxyaporphinium methiodides

them by Hofmann elimination, with free hydroxyl at C-1 or C-11, are known to show hydroxyl frequencies below 3300 cm^{-1}.[190] Alternatively, location of a methoxyl at C-2 and/or C-10 was supported by the nmr spectral data. Methoxyls at C-2 and C-10 resonate at 3.85-390 ppm whereas methoxyls at C-1 and C-11 do so at 3.4-3.7 ppm.[191,192] The methoxyl signals of the Magnolia alkaloid (3.70, 3.90, 3.92 ppm) indicated that the C-2 and C-10 positions had the methoxyl groups.

To discriminate between the structures 4.13 and 4.14 a more detailed comparison of the spectra was made. Although the mass spectra of 4.13 and 4.14 were somewhat different,[189] the spectrum of the Magnolia alkaloid was not sufficiently similar to either of the literature spectra to allow an assignment. Neither were the reported nmr spectra of use:

H-8 and H-9 of 4.13 have been reported as singlets at 7.27 and 7.33 ppm, with H-3 at 7.05 ppm[193] while 4.14 showed H-8 and H-9 as a singlet at 6.96 ppm, and H-3 at 7.02 ppm.[194] As the chemical shift differences for H-8 and H-9 are small, the outer peaks of the AB-quartet are small and, therefore, often overlooked. Furthermore, variations from these values are also found in the literature.[195,196] The spectra also vary depending on the solvent used (D_6DMSO, CD_3OD, CF_3COOH or D_2O). The observation of an AB-quartet for H-8 and H-9 is the key to assignment of all the resonances, whereas, if only singlets appear, the assignments are questionable.

The assignments proposed for the Magnolia alkaloid and its methyl ether based upon the AB-quartets are shown in Table 4.1.

Table 4.1 Spectral assignments of the aromatic protons of Magnolia toxic alkaloid and its ether

	H3, ppm	H8	and	H9
alkaloid	6.96	6.98d	or	7.11d
methyl ether	6.96	6.97d	or	7.15d

The discrimination between 4.13 and 4.14 was ultimately made chemically: in base, N-methylisocorydine 4.14 has an unsubtituted position para to the phenolate function which is very reactive toward electrophilic reagents, whereas N-methylcorydine 4.13 has none. This difference has been exploited in a number of ways: deuteration,[195] and the Gibbs test (reaction with 2,6-dibromoquinone chlorimide)[197]

having been used for this purpose. In this work, diazonium coupling was employed. The thin-layer chromatogram of magnoflorine 4.10, the Magnolia alkaloid, and its methyl ether was sprayed with a solution of 4-carbethoxybenzenediazonium fluoborate, followed by 1% $NaHCO_3$. Magnoflorine and the Magnolia alkaloid both formed highly colored azo dyes, whereas the methyl ether, which has no free-phenolate activating group, did not. Therefore, the Magnolia alkaloid corresponds to 4.14, N-methylisocorydinium iodide, which has a reactive para-position. Further verification was obtained by carrying out the coupling in solution. The presence of a phenolic function conjugated with the azo group was verified by demonstration of a shift in λ_{max} upon alkalinization (375 nm $\rightarrow$ 516 nm). Therefore, it may be concluded that the toxic alkaloid is identical to (+)-N-methyl isocorydinium iodide 4.14.

The present work describes the first reported isolation of (+)-N-methyl isocorydine from any species of Magnolia. N-methyl isocorydine, also known as menisperine, was first isolated from Xanthoxylum brachyanthum F. Muell. and X. veneficum F. M. Bail., two members of Rutaceae by Cannon et al.,[197] who also determined its structure. An alkaloid originally called "Chakranine" isolated from Bragantia wallichii R. Br. (Sanskrit: "Chakrani," Aristolochiaceae)[93] has been found to be identical to N-methyl isocorydinium iodide.[190] The alkaloid is of widespread occurrence, having been isolated from the Annonaceae, Lauraceae, Menispermaceae, Papaveraceae, and Berberidaceae.[191-92] It has recently been synthesized de noveau by a route involving preparation of the corresponding benzyltetrahydroisoquinoline with an amino function at the ortho position of the benzyl

moiety, followed by Pshorr synthesis of the aporphine (closure via the diazonium ion).[198,199]

Non-toxic Alkaloidal Fraction

The second alkaloid isolated mainly from the chloroform extract and described earlier, was obtained as the hydrochloride, a colorless, crystalline solid, mp 265-275°C (dec), $[\alpha]_D$-73° (c 1.1,EtOH). The free base could not be induced to crystallize.

Elemental analysis of the hydrochloride and the mass spectrum (M^+ 265) of the base were consistent with the molecular formula $C_{17}H_{15}NO_2$ for the base. The uv spectrum λ_{max} 235 nm, log ε 4.21; 271 nm, log ε 4.21; 315 nm, log ε 3.75, unchanged in base is characteristic of the aporphine nucleus.[186,187] The nmr spectrum of the free base indicated the presence of a methylenedioxy group (6.00 ppm, 1H, d and 5.87 ppm, 1H d), five aromatic protons: 8.00 ppm, 1H, m, 7.02-7.13, 3H, m, 6.50, 1H, s and other signals characteristic of an aporphine system: 3.7-4.1 ppm, 1H, m (H-6a)[194] and 2.4-3.5 ppm, 6H, m. The presence of a one proton singlet at 2.87 ppm which was shifted by acid, and the lack of a N-methyl function indicated that the nitrogen is secondary. This was verified by the formation of an acetamide, mp 229-231°C.

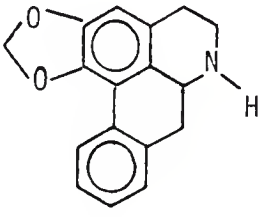
The mass spectrum showed the expected intense molecular ion (m/e 265, 63%) and even stronger M^+ -1 (m/e 264, 100%) characteristic of aporphines.[185,188] Lacking any other functionality except the methylenedioxy group it could not undergo the methoxyl group processes outlined earlier. The only other meaningful ions in the spectra might

be attributed to loss of ·HC=O and H_2C=O (m/e 236, 24% and m/e 235, 14%); and M-31, which might be expected to arise from loss of methylene• imine (retro Diels-Alder) as shown in Figure 4.4.

The nonequivalency of the methylenedioxy signals, which originates from anisotropy due to the twisted biphenyl system, is observed in aporphines bearing the group at the 1,2- or 9,10-positions,[200] i.e., in a position where the methylenes are subject to differential deshielding by the adjacent ring of the biphenyl system.

The only structure which was considered likely for this alkaloid on a biogenetic basis is anonaine 4.16, with a 1,2-methylenedioxy group. A 2,3-methylenedioxy group can be firmly excluded on the basis of the nmr spectrum. Such a group would show a single resonance for the reasons discussed above. The nmr signal at 6.50 ppm was assignable to H-3 and the multiplet at 8.00, deshielded by the adjacent aromatic ring, was assigned to H-11.

From this information it was concluded that the alkaloid was identical to the norapoephine,(-)-anonaine 4.16. This identification was confirmed by the melting point of the acetamide, 229-30°C (lit. 229-30°).[201]



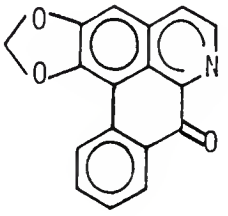
4.16

Anonaine was first isolated by Santos from Annona reticulata L.,[202] but was not identified until 1939 by Barger and Weitnauer.[201] (The difference in spelling is due to the use of the native Philippine name by Santos.) It occurs in Magnolia obovata[35] and M. grandiflora[40] (cf. Table 1.3). Its acetamide occurs in Magnolia obovata[41] and Liriodendron tulipifera L.[203] (Magnoliaceae), a plant which produces a number of acetylated noraporphines.[203,204] It occurs in most species of Annona, and is of very frequent occurrence within the Annonaceae in general.[191,192] Outside this family, it has been reported only from Doryphora sassafrass Endlicher (Monimiaceae),[88] Roemeria refracta DC.[205] (Papaveraceae), Colubrina faralolatra H. Perrier[206] (Rhamnaceae), and Nelumbo nucifera Gaertn.[207,208] (Nymphaceae). In the latter, it is accompanied by 4,5-dehydroanonaine.[208]

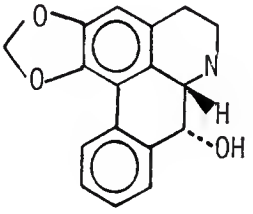
Anonaine was accompanied in this fraction by two minor alkaloids. The nmr spectra suggested that they were the epimeric 7-hydroxyanonaines, but insufficient quantities were available for complete characterization.

The fluorescent, yellow alkaloid occurring in the same fraction was separated from anonaine by extraction and alumina chromatography. On crystallization, 100 mg/5 kg of wood of ochre-colored crystals, mp 280-282°C (dec.) were obtained. The mass spectrum indicated a molecular weight of 275, corresponding to $C_{17}H_9NO_3$. The ir spectrum showed a carbonyl group at 1660 cm^{-1} indicating that the compound was a 7-oxoaporphine. The nmr spectrum showed a methylenedioxy group 6.72, 2H, s, and aromatic protons at 7.63 ppm, 1H, s, and 7.7-8.9, 6H,

m. The uv spectrum showed λ_{max} 246 nm, 265 nm, 280 (sh) nm, and 300 nm. On the basis of the methylenedioxy signal, the unusual chromophore, and the ketonic function, the ir frequency of which is the region associated with a diaryl ketone or amide, the compound was readily identified as liriodenine, 4.17.



4.17



4.18

Liriodenine occurs in a number of Magnolia spp. (cf. Table 1.3), and, like anonaine, is of widespread occurrence throughout the Annonaceae.[191,192] Generally, liriodenine is present in plants containing anonaine. Also known as oxoushinsunine, it often occurs along with ushinsunine 4.18, which may be regarded as an oxidation state intermediate between the oxoaporphines and the aporphines. A second oxoaporphine, oxoglaucine, also occurs in Magnolia (cf. Table 1.3).

Experimental

Isolation of Alkaloid Fractions from Magnolia Wood

Shavings from Magnolia trunk wood (4.9 kg) were extracted with ethanol (12 ℓ) at 20°C for two days. A combination of three such extracts was concentrated to a syrupy consistency (23 g), at which stage it showed an LD_{50} value 700 mg/kg (for details of the biological

assay: cf: Table 1.5). The concentrate was partitioned between ethyl acetate and HCL (0.01N, 800 ml each). The aqueous layer, which contained 28.8 g of total solids with an LD_{50} of 350 mg/kg, was washed once more with ethyl acetate, filtered through cotton to remove any tarry residue. It was concentrated to approximately 250 ml and treated with Mayer's reagent until precipitation was complete. The precipitate was filtered on a Celite pad and the cake washed with HCl (0.01N). The filter cake was stirred with acetone-water (1:1) and filtered. The filtrate and wash were passed through a column of Dowex-1 (20 g, chloride). The column was washed with 1:1 acetone-water until the washings gave a negative test with Mayer's reagent (after removal of the acetone from the aliquot). The effluent and wash, containing an alkaloidal material (7.65 g), LD_{50} 70 mg/kg, was concentrated to remove the acetone, adjusted to pH 8 with ammonia and extracted twice with chloroform.

The chloroform extract was concentrated to 5-10 ml, diluted with an equal volume of chloroform and treated with methanolic HCl (1N). The alkaloid hydrochloride which precipitated out was filtered and washed with ether; yield 1 g (tlc: R_f 0.6, silica gel, 10% methanol:chloroform); and gave a positive reaction with acidic iodoplatinate reagent. The mother liquors from the hydrochloride were set aside for the separation of the trace components.

The aqueous layer, after the chloroform extraction, was readjusted to pH 7 and extracted twice with n-butanol. The butanol layer yielded 3.3 g of a solid, LD_{50}, 80 mg/kg. Thin-layer chromatography showed that it contained a major alkaloidal component (tlc: R_f 0.8, silica

gel, chloroform:methonol:water:acetic acid, 14:6:1:1), along with a minor amount of the alkaloid seen in the chloroform extract. The aqueous layer from n-butanol extraction was concentrated to near dryness to yield a solid, 3.4 g, LD_{50} 40 mg/kg and containing the same alkaloid as the major component of n-butanol extract.

N-methyl isocorydinium iodide 4.14

The butanol-extractable fraction (3.3 g) was chromatographed on Sephadex LH-20 (200 g). Elution with 10% ethanol:ethyl acetate gave a fraction containing anonaine 4.16, corresponding to 50 mg of the hydrochloride. Elution with 20% ethanol:ethyl acetate gave the toxic fraction, 530 mg, LD_{50} 17 mg/kg. The alkaloid was converted to the iodide by passage through a Dowex-1 (iodide) column, and evaporated to dryness. The product was triturated with ether, and crystallized from methanol, giving 4.14, a colorless, crystalline solid, mp 222-25°C, dec. (lit. 225-30°C);[194] LD_{50} 10 mg/kg; $[\alpha]_D$ +212° (c 1.27, EtOH); uv: 220 nm, log ε 4.76; 270 nm, log ε 4.21; 302 nm, log ε 3.82, shifting to 250 and 354 nm in base (lit. 222/4.09, 271/4.09, 304/3.65),[194] ms (m/e): 341 (M^+ 100%), 376 (M^+ -CH_3, 25%), 310 (M^+ -OCH_3, 20%), 298 (M^+ -$CH_2{=}NCH_3$/21%), 283 (m/e 298-CH_3/43%), 270 (m/e 298-C≡O?/96%); 142 (CH_3I, 74%), 127 (I^+, 52%), 56 (20%); nmr (ppm): 8.67, m, ArOH; 7.00, 1H, aromatic; 6.98 and 7.11, AB-quartet, J = 8 Hz, aromatic; 3.72, 3.85, and 3.93, 3H each, s, (OCH_3); 3.44 and 2.98 ppm, 3H each, s, $N(CH_3)_2^+$; ir (KBr): 3200 cm^{-1} (ArOH), 1600 cm^{-1}, aromatic.

Chromatography of the non-extractable fraction, eluted by 20% ethanol: ethyl acetate, afforded 819 mg of 4.14 on workup as before.

Chromatography by partition on a cellulose column, gave much better elution patterns and was easier to carry out: Cellulose (Solka Floc, 200 g) was taken up in ethyl acetate: n-butanol (500 ml, 3:1) equilibrated with water (100 ml), and the mixture stirred until the aqueous phase was no longer apparent (30 min.). The slurry was packed in a column and charged with 10 ml of slurry containing the non-extractable alkaloid fraction (2.3 g), and eluted with the ethyl acetate:butanol system, affording 660 mg of 4.14 iodide on workup.

N-methyl isocorydine methyl ether

A sample of 4.14 (100 mg) was stirred with dimethyl sulfate (0.8 ml) and anhydrous potassium carbonate (0.57 g) in acetone (50 ml) for 48 hours. Tlc showed a single band, R_5 0.50,[a] silica, chloroform: methanol: water, 6:4:1; uv: no shift in base. The product was filtered, the cake washed with ether, the product leached out with methanol, and converted to the iodide as before. Crystallization from methanol gave the methyl ether iodide 4.14, 100 mg, mp 255°C, dec. (lit. 258°C, dec.);[197] uv: similar to 4.14, but not shifted in base; ir: 3200 cm^{-1} no longer present; nmr (ppm): 3.71, 6H, 3.90, 3H, 3.92, 3H; ms: M+ 369).

Magnoflorine dimethyl ether 4.11

A sample of 4.10 (100 mg), dimethyl sulfate (1.6 ml) and anhydrous potassium carbonate (1.2 g) was methylated and worked up as described for 4.14 methyl ether, given 4.11, 100 mg, identical to 4.14 methyl ether by tlc, ir, and nmr spectral comparison.

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[a]Starting material, R_f 0 in this system.

O-Acetyl-N-methyl isocorydine

A sample of 4.14 (100 mg) in acetic anhydride (5 m) was stirred overnight with pyridine (0.1 ml). Tlc showed no reaction, so more pyridine (5 ml) was added. After 48 hours, no change was noted (tlc). Heating at 50°C for two hours also failed to cause acetylation.

In acetic anhydride (5 ml) containing one drop of phosphoric acid, 4.14 (100 mg) was successfully acetylated by heating two hours at 100°C (tlc: R_f 0.8, silica, chloroform:methanol:water 14:16:1). The product was cooled, dilutes with methanol and passed through a Dowex-1 (acetate) column to remove phosphoric acid. The product was concentrated to dryness and chromatographed (Sephadex LH-20 g, eluted by 10% ethanol: ethyl acetate) then converted to the iodide as before. Crystallization from methanol gave O-acetyl 4.14 iodide, 70 mg, mp 265°C, dec. (lit. 260-265°C, dec.):[194] ir: 1770 cm^{-1} (CH_3CO_2Ar) no 3200 cm^{-1} band; nmr: 2.10 ppm.

Anonaine Hydrochloride 4.14

The chlorofom extractable alkaloids and the chromatographic fraction from the toxic, butanol fraction, were taken up in chloroform (5 ml) and 4.16 hydrochloride precipitated by careful addition of methanolic HCl (1N). The mother liquors were chromatographed on alumina (20 g) with 5% methanol:chloroform, to afford a total of 1 g of 4.16 on conversion to the salt. Several recrystallizations from methanol gave mp 265-275°C, dec. (lit. 277.5°C, dec.);[202] $[\alpha]_D$-73° (c 1.1,EtOH) (lit. -69°)[208] uv: 235 nm, log 4.21; 271 nm, log 4.21; 315 nm, log 3.75 (lit. 234 nm/4.24, 269 nm/4.24, 312 nm/3.49);[203] nmr (ppm, free base in $CDCl_3$): 6.00 and 5.87 ppm, two 1H d, J = 1.5 Hz, methylenedioxy, five aromatic

protons: 8.00, 1H, m; 7.02-7.13, 3H, m; 6.50, 1H, s; in addition to 3.7-4.1, 1H, m (H-6a); 2.4-3.5 ppm, 6H, m; and 2.87 ppm, 1H, s, NH; ms (m/e): 265 (M^+ 63%), 264 (M^+ -1, 100%), 236 (M^+ -29), 234 (M^+ -31).

Anal. Calc. for $C_{17}H_{16}NO_2Cl \cdot \frac{1}{2} H_2O$: C, 65.70; H, 5.51; N, 4.51; Cl, 11.41. Found: C, 66.61; H, 5.23; N, 4.57; Cl, 11.40.

Anonaine acetamide

In acetic anhydride:acetic acid (5 ml, 1:1) 4.16 (100 mg) was stirred overnight, then heated at 70°C for 30 min. The reaction mixture was poured over ice and the product filtered to give 4.16 acetamide (100 mg). Additional material was obtained by adjusting the filtrate to pH 9, and extracting with chloroform. The chloroform extracts were washed with 10% sodium bicarbonate, and dried (Na_2SO_4). Concentration gave 10 mg 4.14, tlc: R_f 4.16, 0.60; amide 0.80, silica, 10% methanol: chloroform, the amide giving no reaction with acidic iodoplatinate reagent. Crystallization from methanol gave 100 mg, mp 229-31°C (lit. 229-30°C);[203] ir: 1645 cm^{-1} (lit. 164);[203] nmr ($CDCl_3$): 2.21, 3H, s (CH_3CON).

Liriodenine 4.17

Weakly basic 4.17 was isolated during chromatography of 4.16 (tlc: 4.16, R_f 0.60, 4.17, R_f 0.95, silica, 10% methanol (chloroform) and from liquors of crystallization of 4.16. After extracting 4.16 and trace alkaloids with 9.01 N HCl, 4.17 was extracted into 1N HCl, neutralized, and reextracted into chloroform. On concentration 4.17 was obtained as an ochre powder, recrystallization from chloroform giving mp 280-2°C, dec.

(lit. 285-6°C);[209] ir: 1655 cm^{-1} (lit. 1660 cm^{-1});[209] nmr (ppm): 6.72, 2H, s, methylenedioxy, aromatic protons: 7.63, 1H, s, and 7.7-8.9, 6H, m; uv: 246 nm, 265 nm, 280 nm (sh), and 300 nm (lit. 245 nm; 265 nm, 280 nm).[209]

General Experimental

Melting points were obtained with a Fisher-Johns (hot-stage) apparatus and are uncorrected. Infrared (ir) spectra were recorded with a Beckman Acculab 3 spectrophotometer as KBr pellets unless otherwise indicated. Ultraviolet (uv) spectra were obtained with a Beckman DB UV/visible spectrophotometer using a Sargent Model SRL recorder. Nuclear magnetic resonance (nmr) spectra were obtained with a Varian A60-A or T60-A spectrometers, with a tetramethyl silane (TMS) internal standard. High-resolution nmr spectra were determined by Dr. Wallace Brey of the Department of Chemistry of the University of Florida, employing a Varian HA-100 spectrometer. Alkaloid spectra are in D_6DMSO unless otherwise specified. Mass spectra (ms) were obtained using an Hitachi-Perkin-Elmer RMU-6E, single-focusing, electron impact spectrometer, with a Perkin-Elmer MS-30 double-focusing spectrometer being used for high-resolution work. Thin-layer chromatography (tlc) plates were hand-cast on microscope slides with E. Merck silica gel PF 254+366 without binder. Gas chromatography (gc) was carried out using a Varian series 2100 gas chromatograph with flame-ionization detector. Silanized glass columns, six feet in length and 3/16 inch i.d. were routinely employed. Integration of peak areas was carried out by triangulation, and quantitative figures are uncorrected for flame-sensitivity factors. Standard flow

rates were: hydrogen, 40 ml/min.; air, 300 ml/min.; and helium, 60 ml/min. Molecular rotations were obtained with a Perkin-Elmer model 141 polarimeter.

Antibiotic assays were carried out using the filter-paper disk method on agar seeded with Bacillus subtilis spore. The agar medium consisted of 1% (w/v) dextrose, 1.8% agar (Difco Bacteriological), 0.3% peptone (Difco, Technical), and 0.3% beef extract (Difco, Technical). The agar was autoclaved and cooled in a bath to ca. 55°C before inoculation with 0.5 ml/200 ml of medium of B. subtilis spore suspension prepared according to the method of Grove and Randall.[210] The agar was poured in 10 ml aliquots into Petri dishes and stored under refrigeration. Filter-paper disks (Schleicher and Schuell No. 740-E) were impregnated with test solutions and plated. The petri dishes were incubated 24 hrs. at 37°C and the clear zone of inhibition read. Minimal inhibitory concentrations (MIC) were determined by serial dilution, plotting log concentration vs. diameter of the zone of inhibition, and extrapolating to the origin.

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BIOGRAPHICAL SKETCH

Terry Lee Davis was born in Oak Ridge, Tennessee, on October 31, 1945, the oldest of three sons. His family moved to Louisville, Kentucky, in 1950. He graduated from Seneca High School in 1963.

While attending the University of Kentucky, he was a member of Alpha Tau Omega social fraternity and in 1966 was president of the University of Kentucky chapter of Alpha Chi Sigma, chemical fraternity. He received his B.S. degree from the University of Kentucky in 1967.

He married his wife, Sandi, in June of 1969. Sandi is a teacher, with a master's degree in early childhood education. They have one daughter, Tiffany, born in December 1976.

While attending the University of Florida, he received a teaching award from the department of chemistry in 1970. He received the master's degree in chemistry in June 1971.

Terry's interests are bonsai and exotic plants. He has published a number of articles on bonsai.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David N. Silverman
Professor of Pharmacology and Therapeutics

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John A. Zoltewicz
Professor of Chemistry

This dissertation was submitted to the Graduate Faculty of the College of Pharmacy and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 1981

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